# UNITED STATES AIR FORCE

# SUMMER RESEARCH PROGRAM -- 1997

# HIGH SCHOOL APPRENTICESHIP PROGRAM
# FINAL REPORTS

## VOLUME 12B

## ARMSTRONG LABORATORY


RESEARCH & DEVELOPMENT LABORATORIES

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The United States Air Force Summer Research Program (USAF-SRP) is designed to introduce university, college, and technical institute faculty members, graduate students, and high school students to Air Force research. This is accomplished by the faculty members (Summer Faculty Research Program, (SFRP)), graduate students (Graduate Student Research Program (GSRP)), and high school students (High School Apprenticeship Program (HSAP)) being selected on a nationally advertised competitive basis during the summer intersession period to perform research at Air Force Research Laboratory (AFRL) Technical Directorates, Air Force Air Logistics Centers (ALC), and other AF Laboratories. This volume consists of a program overview, program management statistics, and the final technical reports from the HSAP participants at the Armstrong Laboratory.

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## PREFACE

Reports in this volume are numbered consecutively beginning with number 1. Each report is paginated with the report number followed by consecutive page numbers, e.g., 1-1, 1-2, 1-3; 2-1, 2-2, 2-3.

Due to its length, Volume 12A is bound in two parts, 12A and 12B. Volume 12A contains #1-20. Volume 12B contains reports #21-41. The Table of Contents for Volume 12 is included in both parts.

This document is one of a set of 16 volumes describing the 1997 AFOSR Summer Research Program. The following volumes comprise the set:

| VOLUME | TITLE |
|---|---|
| 1 | Program Management Report |
| | ***Summer Faculty Research Program (SFRP) Reports*** |
| 2A & 2B | Armstrong Laboratory |
| 3A & 3B | Phillips Laboratory |
| 4A & 4B | Rome Laboratory |
| 5A, 5B & 5C | Wright Laboratory |
| 6 | Arrold Engineering Development Center, U.S. Air Force Academy, Air Logistic Centers, and Wilford Hall Medical Center |
| | ***Graduate Student Research Program (GSRP) Reports*** |
| 7A & 7B | Armstrong Laboratory |
| 8 | Phillips Laboratory |
| 9 | Rome Laboratory |
| 10A & 10B | Wright Laboratory |
| 11 | Arrold Engineering Development Center, U.S. Air Force Academy, Air Logistic Centers, and Wilford Hall Medical Center |
| | ***High School Apprenticeship Program (HSAP) Reports*** |
| 12A & 12B | Armstrong Laboratory |
| 13 | Phillips Laboratory |
| 14 | Rome Laboratory |
| 15A&15B | Wright Laboratory |
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# 1. INTRODUCTION

The Summer Research Program (SRP), sponsored by the Air Force Office of Scientific Research (AFOSR), offers paid opportunities for university faculty, graduate students, and high school students to conduct research in U.S. Air Force research laboratories nationwide during the summer.

Introduced by AFOSR in 1978, this innovative program is based on the concept of teaming academic researchers with Air Force scientists in the same disciplines using laboratory facilities and equipment not often available at associates' institutions.

The Summer Faculty Research Program (SFRP) is open annually to approximately 150 faculty members with at least two years of teaching and/or research experience in accredited U.S. colleges, universities, or technical institutions. SFRP associates must be either U.S. citizens or permanent residents.

The Graduate Student Research Program (GSRP) is open annually to approximately 100 graduate students holding a bachelor's or a master's degree; GSRP associates must be U.S. citizens enrolled full time at an accredited institution.

The High School Apprentice Program (HSAP) annually selects about 125 high school students located within a twenty mile commuting distance of participating Air Force laboratories.

AFOSR also offers its research associates an opportunity, under the Summer Research Extension Program (SREP), to continue their AFOSR-sponsored research at their home institutions through the award of research grants. In 1994 the maximum amount of each grant was increased from $20,000 to $25,000, and the number of AFOSR-sponsored grants decreased from 75 to 60. A separate annual report is compiled on the SREP.

The numbers of projected summer research participants in each of the three categories and SREP "grants" are usually increased through direct sponsorship by participating laboratories.

AFOSR's SRP has well served its objectives of building critical links between Air Force research laboratories and the academic community. opening avenues of communications and forging new research relationships between Air Force and academic technical experts in areas of national interest, and strengthening the nation's efforts to sustain careers in science and engineering. The success of the SRP can be gauged from its growth from inception (see Table 1) and from the favorable responses the 1997 participants expressed in end-of-tour SRP evaluations (Appendix B).

AFOSR contracts for administration of the SRP by civilian contractors. The contract was first awarded to Research & Development Laboratories (RDL) in September 1990. After completion of the

1990 contract, RDL (in 1993) won the recompetition for the basic year and four 1-year options.

## 2. PARTICIPATION IN THE SUMMER RESEARCH PROGRAM

The SRP began with faculty associates in 1979; graduate students were added in 1982 and high school students in 1986. The following table shows the number of associates in the program each year.

| YEAR | SRP Participation, by Year | | | TOTAL |
|---|---|---|---|---|
| | SFRP | GSRP | HSAP | |
| 1979 | 70 | | | 70 |
| 1980 | 87 | | | 87 |
| 1981 | 87 | | | 87 |
| 1982 | 91 | 17 | | 108 |
| 1983 | 101 | 53 | | 154 |
| 1984 | 152 | 84 | | 236 |
| 1985 | 154 | 92 | | 246 |
| 1986 | 158 | 100 | 42 | 300 |
| 1987 | 159 | 101 | 73 | 333 |
| 1988 | 153 | 107 | 101 | 361 |
| 1989 | 168 | 102 | 103 | 373 |
| 1990 | 165 | 121 | 132 | 418 |
| 1991 | 170 | 142 | 132 | 444 |
| 1992 | 185 | 121 | 159 | 464 |
| 1993 | 187 | 117 | 136 | 440 |
| 1994 | 192 | 117 | 133 | 442 |
| 1995 | 190 | 115 | 137 | 442 |
| 1996 | 188 | 109 | 138 | 435 |
| 1997 | 148 | 98 | 140 | 427 |

Beginning in 1993, due to budget cuts, some of the laboratories weren't able to afford to fund as many associates as in previous years. Since then, the number of funded positions has remained fairly constant at a slightly lower level.

## 3. RECRUITING AND SELECTION

The SRP is conducted on a nationally advertised and competitive-selection basis. The advertising for faculty and graduate students consisted primarily of the mailing of 8,000 52-page SRP brochures to chairpersons of departments relevant to AFOSR research and to administrators of grants in accredited universities, colleges, and technical institutions. Historically Black Colleges and Universities (HBCUs) and Minority Institutions (MIs) were included. Brochures also went to all participating USAF laboratories, the previous year's participants, and numerous individual requesters (over 1000 annually).

RDL placed advertisements in the following publications: *Black Issues in Higher Education, Winds of Change,* and *IEEE Spectrum.* Because no participants list either *Physics Today* or *Chemical & Engineering News* as being their source of learning about the program for the past several years, advertisements in these magazines were dropped, and the funds were used to cover increases in brochure printing costs.

High school applicants can participate only in laboratories located no more than 20 miles from their residence. Tailored brochures on the HSAP were sent to the head counselors of 180 high schools in the vicinity of participating laboratories, with instructions for publicizing the program in their schools. High school students selected to serve at Wright Laboratory's Armament Directorate (Eglin Air Force Base, Florida) serve eleven weeks as opposed to the eight weeks normally worked by high school students at all other participating laboratories.

Each SFRP or GSRP applicant is given a first, second, and third choice of laboratory. High school students who have more than one laboratory or directorate near their homes are also given first, second, and third choices.

Laboratories make their selections and prioritize their nominees. AFOSR then determines the number to be funded at each laboratory and approves laboratories' selections.

Subsequently, laboratories use their own funds to sponsor additional candidates. Some selectees do not accept the appointment, so alternate candidates are chosen. This multi-step selection procedure results in some candidates being notified of their acceptance after scheduled deadlines. The total applicants and participants for 1997 are shown in this table.

| 199? Applicants and Participants | | | |
|---|---|---|---|
| PARTICIPANT CATEGORY | TOTAL APPLICANTS | SELECTEES | DECLINING SELECTEES |
| SFRP | 490 | 188 | 32 |
| (HBCU/MI) | ( 0 ) | ( 0 ) | ( 0 ) |
| GSRP | 202 | 98 | 9 |
| (HBCU/MI) | ( 0 ) | ( 0 ) | ( 0 ) |
| HSAP | 433 | 140 | 14 |
| TOTAL | 1125 | 426 | 55 |

## 4. SITE VISITS

During June and July of 1997, representatives of both AFOSR/NI and RDL visited each participating laboratory to provide briefings, answer questions. and resolve problems for both laboratory personnel and participants. The objective was to ensure that the SRP would be as constructive as possible for all participants. Both SRP participants and RDL representatives found these visits beneficial. At many of the laboratories, this was the only opportunity for all participants to meet at one time to share their experiences and exchange ideas.

## 5. HISTORICALLY BLACK COLLEGES AND UNIVERSITIES AND MINORITY INSTITUTIONS (HBCU/MIs)

Before 1993, an RDL program representative visited from seven to ten different HBCU/MIs annually to promote interest in the SRP among the faculty and graduate students. These efforts were marginally effective, yielding a doubling of HBCI/MI applicants. In an effort to achieve AFOSR's goal of 10% of all applicants and selectees being HBCU/MI qualified, the RDL team decided to try other avenues of approach to increase the number of qualified applicants. Through the combined efforts of the AFOSR Program Office at Bolling AFB and RDL, two very active minority groups were found, HACU (Hispanic American Colleges and Universities) and AISES (American Indian Science and Engineering Society). RDL is in communication with representatives of each of these organizations on a monthly basis to keep up with the their activities and special events. Both organizations have widely-distributed magazines/quarterlies in which RDL placed ads.

Since 1994 the number of both SFRP and GSRP HBCU/MI applicants and participants has increased ten-fold, from about two dozen SFRP applicants and a half dozen selectees to over 100 applicants and two dozen selectees, and a half-dozen GSRP applicants and two or three selectees to 18 applicants and 7 or 8 selectees. Since 1993, the SFRP had a two-fold applicant increase and a two-fold selectee increase. Since 1993, the GSRP had a three-fold applicant increase and a three to four-fold increase in selectees.

In addition to RDL's special recruiting efforts, AFOSR attempts each year to obtain additional funding or use leftover funding from cancellations the past year to fund HBCU/MI associates. This year, 5 HBCU/MI SFRPs declined after they were selected (and there was no one qualified to replace them with). The following table records HBCU/MI participation in this program.

| SRP HBCU/MI Participation, By Year | | | | |
|---|---|---|---|---|
| YEAR | SFRP | | GSRP | |
| | Applicants | Participants | Applicants | Participants |
| 1985 | 76 | 23 | 15 | 11 |
| 1986 | 70 | 18 | 20 | 10 |
| 1987 | 82 | 32 | 32 | 10 |
| 1988 | 53 | 17 | 23 | 14 |
| 1989 | 39 | 15 | 13 | 4 |
| 1990 | 43 | 14 | 17 | 3 |
| 1991 | 42 | 13 | 8 | 5 |
| 1992 | 70 | 13 | 9 | 5 |
| 1993 | 60 | 13 | 6 | 2 |
| 1994 | 90 | 16 | 11 | 6 |
| 1995 | 90 | 21 | 20 | 8 |
| 1996 | 119 | 27 | 18 | 7 |

## 6. SRP FUNDING SOURCES

Funding sources for the 1997 SRP were the AFOSR-provided slots for the basic contract and laboratory funds. Funding sources by category for the 1997 SRP selected participants are shown here.

| 1997 SRP FUNDING CATEGORY | SFRP | GSRP | HSAP |
|---|---|---|---|
| AFOSR Basic Allocation Funds | 141 | 89 | 123 |
| USAF Laboratory Funds | 48 | 9 | 17 |
| HBCU/MI By AFOSR (Using Procured Addn'l Funds) | 0 | 0 | N/A |
| TOTAL | 9 | 98 | 140 |

SFRP - 188 were selected, but thirty two canceled too late to be replaced.
GSRP - 98 were selected, but nine canceled too late to be replaced.
HSAP - 140 were selected, but fourteen canceled too late to be replaced.

## 7. COMPENSATION FOR PARTICIPANTS

Compensation for SRP participants, per five-day work week, is shown in this table.

1997 SRP Associate Compensation

| PARTICIPANT CATEGORY | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 |
|---|---|---|---|---|---|---|---|
| Faculty Members | $690 | $718 | $740 | $740 | $740 | $770 | $770 |
| Graduate Student (Master's Degree) | $425 | $442 | $455 | $455 | $455 | $470 | $470 |
| Graduate Student (Bachelor's Degree) | $365 | $380 | $391 | $391 | $391 | $400 | $400 |
| High School Student (First Year) | $200 | $200 | $200 | $200 | $200 | $200 | $200 |
| High School Student (Subsequent Years) | $240 | $240 | $240 | $240 | $240 | $240 | $240 |

The program also offered associates whose homes were more than 50 miles from the laboratory an expense allowance (seven days per week) of $50/day for faculty and $40/day for graduate students. Transportation to the laboratory at the beginning of their tour and back to their home destinations at the end was also reimbursed for these participants. Of the combined SFRP and GSRP associates, 65 % (194 out of 286) claimed travel reimbursements at an average round-trip cost of $776.

Faculty members were encouraged to visit their laboratories before their summer tour began. All costs of these orientation visits were reimbursed. Forty-three percent (85 out of 188) of faculty associates took orientation trips at an average cost of $388. By contrast, in 1993, 58 % of SFRP associates took

orientation visits at an average cost of $685; that was the highest percentage of associates opting to take an orientation trip since RDL has administered the SRP, and the highest average cost of an orientation trip. These 1993 numbers are included to show the fluctuation which can occur in these numbers for planning purposes.

Program participants submitted biweekly vouchers countersigned by their laboratory research focal point, and RDL issued paychecks so as to arrive in associates' hands two weeks later.

This is the second year of using direct deposit for the SFRP and GSRP associates. The process went much more smoothly with respect to obtaining required information from the associates, only 7% of the associates' information needed clarification in order for direct deposit to properly function as opposed to 10% from last year. The remaining associates received their stipend and expense payments via checks sent in the US mail.

HSAP program participants were considered actual RDL employees, and their respective state and federal income tax and Social Security were withheld from their paychecks. By the nature of their independent research, SFRP and GSRP program participants were considered to be consultants or independent contractors. As such, SFRP and GSRP associates were responsible for their own income taxes, Social Security, and insurance.

## 8. CONTENTS OF THE 1997 REPORT

The complete set of reports for the 1997 SRP includes this program management report (Volume 1) augmented by fifteen volumes of final research reports by the 1997 associates, as indicated below:

1997 SRP Final Report Volume Assignments

| LABORATORY | SFRP | GSRP | HSAP |
|---|---|---|---|
| Armstrong | 2 | 7 | 12 |
| Phillips | 3 | 8 | 13 |
| Rome | 4 | 9 | 14 |
| Wright | 5A, 5B | 10 | 15 |
| AEDC, ALCs, WHMC | 6 | 11 | 16 |

## APPENDIX A -- PROGRAM STATISTICAL SUMMARY

A. Colleges/Universities Represented

Selected SFRP associates represented 169 different colleges, universities, and institutions. GSRP associates represented 95 different colleges, universities, and institutions.

B. States Represented

SFRP -Applicants came from 47 states plus Washington D.C. Selectees represent 44 states.

GSRP - Applicants came from 44 states. Selectees represent 32 states.

HSAP - Applicants came from thirteen states. Selectees represent nine states.

| Total Number of Participants | |
|---|---|
| SFRP | 189 |
| GSRP | 97 |
| HSAP | 140 |
| TOTAL | 426 |

| Degrees Represented | | | |
|---|---|---|---|
| | SFRP | GSRP | TOTAL |
| Doctoral | 184 | 0 | 184 |
| Master's | 2 | 41 | 43 |
| Bachelor's | 0 | 56 | 56 |
| TOTAL | 186 | 97 | 298 |

| SFRP Academic Titles | |
|---|---|
| Assistant Professor | 64 |
| Associate Professor | 70 |
| Professor | 40 |
| Instructor | 0 |
| Chairman | 1 |
| Visiting Professor | 1 |
| Visiting Assoc. Prof. | 1 |
| Research Associate | 9 |
| TOTAL | 186 |

| Source of Learning About the SRP | | |
|---|---|---|
| Category | Applicants | Selectees |
| Applied/participated in prior years | 28% | 34% |
| Colleague familiar with SRP | 19% | 16% |
| Brochure mailed to institution | 23% | 17% |
| Contact with Air Force laboratory | 17% | 23% |
| *IEEE Spectrum* | 2% | 1% |
| *BIIHE* | 1% | 1% |
| Other source | 10% | 8% |
| TOTAL | 100% | 100% |

## APPENDIX B – SRP EVALUATION RESPONSES

### 1. OVERVIEW

Evaluations were completed and returned to RDL by four groups at the completion of the SRP. The number of respondents in each group is shown below.

Table B-1. Total SRP Evaluations Received

| Evaluation Group | Responses |
|---|---|
| SFRP & GSRPs | 275 |
| HSAPs | 113 |
| USAF Laboratory Focal Points | 84 |
| USAF Laboratory HSAP Mentors | 6 |

All groups indicate unanimous enthusiasm for the SRP experience.

The summarized recommendations for program improvement from both associates and laboratory personnel are listed below:

A. Better preparation on the labs' part prior to associates' arrival (i.e., office space, computer assets, clearly defined scope of work).

B. Faculty Associates suggest higher stipends for SFRP associates.

C. Both HSAP Air Force laboratory mentors and associates would like the summer tour extended from the current 8 weeks to either 10 or 11 weeks; the groups state it takes 4-6 weeks just to get high school students up-to-speed on what's going on at laboratory. (Note: this same argument was used to raise the faculty and graduate student participation time a few years ago.)

## 2. 1997 USAF LABORATORY FOCAL POINT (LFP) EVALUATION RESPONSES

The summarized results listed below are from the 84 LFP evaluations received.

1. LFP evaluations received and associate preferences:

Table B-2. Air Force LFP Evaluation Responses (By Type)

| Lab | Evals Recv'd | How Many Associates Would You Prefer To Get? SFRP 0 | SFRP 1 | SFRP 2 | SFRP 3+ | GSRP (w/Univ Professor) 0 | GSRP (w/Univ Professor) 1 | GSRP (w/Univ Professor) 2 | GSRP (w/Univ Professor) 3+ | (% Response) GSRP (w/o Univ Professor) 0 | GSRP (w/o Univ Professor) 1 | GSRP (w/o Univ Professor) 2 | GSRP (w/o Univ Professor) 3+ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AEDC | 0 | - | - | - | - | - | - | - | - | - | - | - | - |
| WHMC | 0 | - | - | - | - | - | - | - | - | - | - | - | - |
| AL | 7 | 28 | 28 | 28 | 14 | 54 | 14 | 28 | 0 | 86 | 0 | 14 | 0 |
| USAFA | 1 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 0 | 0 | 100 | 0 | 0 |
| PL | 25 | 40 | 40 | 16 | 4 | 88 | 12 | 0 | 0 | 84 | 12 | 4 | 0 |
| RL | 5 | 60 | 40 | 0 | 0 | 80 | 10 | 0 | 0 | 100 | 0 | 0 | 0 |
| WL | 46 | 30 | 43 | 20 | 6 | 78 | 17 | 4 | 0 | 93 | 4 | 2 | 0 |
| Total | 84 | 32% | 50% | 13% | 5% | 80% | 11% | 6% | 0% | 73% | 23% | 4% | 0% |

**LFP Evaluation Summary.** The summarized responses, by laboratory, are listed on the following page. LFPs were asked to rate the following questions on a scale from 1 (below average) to 5 (above average).

2. LFPs involved in SRP associate application evaluation process:
   a. Time available for evaluation of applications:
   b. Adequacy of applications for selection process:
3. Value of orientation trips:
4. Length of research tour:
5. a. Benefits of associate's work to laboratory:
   b. Benefits of associate's work to Air Force:
6. a. Enhancement of research qualifications for LFP and staff:
   b. Enhancement of research qualifications for SFRP associate:
   c. Enhancement of research qualifications for GSRP associate:
7. a. Enhancement of knowledge for LFP and staff:
   b. Enhancement of knowledge for SFRP associate:
   c. Enhancement of knowledge for GSRP associate:
8. Value of Air Force and university links:
9. Potential for future collaboration:
10. a. Your working relationship with SFRP:
    b. Your working relationship with GSRP:
11. Expenditure of your time worthwhile:

(Continued on next page)

12. Quality of program literature for associate:
13. a. Quality of RDL's communications with you:
    b. Quality of RDL's communications with associates:
14. Overall assessment of SRP:

Table B-3. Laboratory Focal Point Reponses to above questions

| | *AEDC* | *AL* | *USAFA* | *PL* | *RL* | *WHMC* | *WL* |
|---|---|---|---|---|---|---|---|
| *# Evals Recv'd* | 0 | 7 | 1 | 14 | 5 | 0 | 46 |
| *Question #* | | | | | | | |
| 2 | - | 86 % | 0 % | 88 % | 80 % | - | 85 % |
| 2a | - | 4.3 | n/a | 3.8 | 4.0 | - | 3.6 |
| 2b | - | 4.0 | n/a | 3.9 | 4.5 | - | 4.1 |
| 3 | - | 4.5 | n/a | 4.3 | 4.3 | - | 3.7 |
| 4 | - | 4.1 | 4.0 | 4.1 | 4.2 | - | 3.9 |
| 5a | - | 4.3 | 5.0 | 4.3 | 4.6 | - | 4.4 |
| 5b | - | 4.5 | n/a | 4.2 | 4.6 | - | 4.3 |
| 6a | - | 4.5 | 5.0 | 4.0 | 4.4 | - | 4.3 |
| 6b | - | 4.3 | n/a | 4.1 | 5.0 | - | 4.4 |
| 6c | - | 3.7 | 5.0 | 3.5 | 5.0 | - | 4.3 |
| 7a | - | 4.7 | 5.0 | 4.0 | 4.4 | - | 4.3 |
| 7b | - | 4.3 | n/a | 4.2 | 5.0 | - | 4.4 |
| 7c | - | 4.0 | 5.0 | 3.9 | 5.0 | - | 4.3 |
| 8 | - | 4.6 | 4.0 | 4.5 | 4.6 | - | 4.3 |
| 9 | - | 4.9 | 5.0 | 4.4 | 4.8 | - | 4.2 |
| 10a | - | 5.0 | n/a | 4.6 | 4.6 | - | 4.6 |
| 10b | - | 4.7 | 5.0 | 3.9 | 5.0 | - | 4.4 |
| 11 | - | 4.6 | 5.0 | 4.4 | 4.8 | - | 4.4 |
| 12 | - | 4.0 | 4.0 | 4.0 | 4.2 | - | 3.8 |
| 13a | - | 3.2 | 4.0 | 3.5 | 3.8 | - | 3.4 |
| 13b | - | 3.4 | 4.0 | 3.6 | 4.5 | - | 3.6 |
| 14 | - | 4.4 | 5.0 | 4.4 | 4.8 | - | 4.4 |

## 3. 1997 SFRP & GSRP EVALUATION RESPONSES

The summarized results listed below are from the 257 SFRP/GSRP evaluations received.

Associates were asked to rate the following questions on a scale from 1 (below average) to 5 (above average) - by Air Force base results and over-all results of the 1997 evaluations are listed after the questions.

1. The match between the laboratories research and your field:
2. Your working relationship with your LFP:
3. Enhancement of your academic qualifications:
4. Enhancement of your research qualifications:
5. Lab readiness for you: LFP, task, plan:
6. Lab readiness for you: equipment, supplies, facilities:
7. Lab resources:
8. Lab research and administrative support:
9. Adequacy of brochure and associate handbook:
10. RDL communications with you:
11. Overall payment procedures:
12. Overall assessment of the SRP:
13. a. Would you apply again?
    b. Will you continue this or related research?
14. Was length of your tour satisfactory?
15. Percentage of associates who experienced difficulties in finding housing:
16. Where did you stay during your SRP tour?
    a. At Home:
    b. With Friend:
    c. On Local Economy:
    d. Base Quarters:
17. Value of orientation visit:
    a. Essential:
    b. Convenient:
    c. Not Worth Cost:
    d. Not Used:

SFRP and GSRP associate's responses are listed in tabular format on the following page.

Table B-4. 1997 SFRP & GSRP Associate Responses to SRP Evaluation

| | Arnold | Brooks | Edwards | Eglin | Griffis | Hanscom | Kelly | Kirtland | Lackland | Robins | Tyndall | WPAFB | average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # res | 6 | 48 | 6 | 14 | 31 | 19 | 3 | 32 | 1 | 2 | 10 | 85 | 257 |
| 1 | 4.8 | 4.4 | 4.6 | 4.7 | 4.4 | 4.9 | 4.6 | 4.6 | 5.0 | 5.0 | 4.0 | 4.7 | 4.6 |
| 2 | 5.0 | 4.6 | 4.1 | 4.9 | 4.7 | 4.7 | 5.0 | 4.7 | 5.0 | 5.0 | 4.6 | 4.8 | 4.7 |
| 3 | 4.5 | 4.4 | 4.0 | 4.6 | 4.3 | 4.2 | 4.3 | 4.4 | 5.0 | 5.0 | 4.5 | 4.3 | 4.4 |
| 4 | 4.3 | 4.5 | 3.8 | 4.6 | 4.4 | 4.4 | 4.3 | 4.6 | 5.0 | 4.0 | 4.4 | 4.5 | 4.5 |
| 5 | 4.5 | 4.3 | 3.3 | 4.8 | 4.4 | 4.5 | 4.3 | 4.2 | 5.0 | 5.0 | 3.9 | 4.4 | 4.4 |
| 6 | 4.3 | 4.3 | 3.7 | 4.7 | 4.4 | 4.5 | 4.0 | 3.8 | 5.0 | 5.0 | 3.8 | 4.2 | 4.2 |
| 7 | 4.5 | 4.4 | 4.2 | 4.8 | 4.5 | 4.3 | 4.3 | 4.1 | 5.0 | 5.0 | 4.3 | 4.3 | 4.4 |
| 8 | 4.5 | 4.6 | 3.0 | 4.9 | 4.4 | 4.3 | 4.3 | 4.5 | 5.0 | 5.0 | 4.7 | 4.5 | 4.5 |
| 9 | 4.7 | 4.5 | 4.7 | 4.5 | 4.3 | 4.5 | 4.7 | 4.3 | 5.0 | 5.0 | 4.1 | 4.5 | 4.5 |
| 10 | 4.2 | 4.4 | 4.7 | 4.4 | 4.1 | 4.1 | 4.0 | 4.2 | 5.0 | 4.5 | 3.6 | 4.4 | 4.3 |
| 11 | 3.8 | 4.1 | 4.5 | 4.0 | 3.9 | 4.1 | 4.0 | 4.0 | 3.0 | 4.0 | 3.7 | 4.0 | 4.0 |
| 12 | 5.7 | 4.7 | 4.3 | 4.9 | 4.5 | 4.9 | 4.7 | 4.6 | 5.0 | 4.5 | 4.6 | 4.5 | 4.6 |
| Numbers below are percentages | | | | | | | | | | | | | |
| 13a | 83 | 90 | 83 | 93 | 87 | 75 | 100 | 81 | 100 | 100 | 100 | 86 | 87 |
| 13b | 100 | 89 | 83 | 100 | 94 | 98 | 100 | 94 | 100 | 100 | 100 | 94 | 93 |
| 14 | 83 | 96 | 100 | 90 | 87 | 80 | 100 | 92 | 100 | 100 | 70 | 84 | 88 |
| 15 | 17 | 6 | 0 | 33 | 20 | 76 | 33 | 25 | 0 | 100 | 20 | 8 | 39 |
| 16a | - | 26 | 17 | 9 | 38 | 23 | 33 | 4 | - | - | - | 30 | |
| 16b | 100 | 33 | - | 40 | - | 8 | - | - | - | - | 36 | 2 | |
| 16c | - | 41 | 83 | 40 | 62 | 69 | 67 | 96 | 100 | 100 | 64 | 68 | |
| 16d | - | - | - | - | - | - | - | - | - | - | - | 0 | |
| 17a | - | 33 | 100 | 17 | 50 | 14 | 67 | 39 | - | 50 | 40 | 31 | 35 |
| 17b | - | 21 | - | 17 | 10 | 14 | - | 24 | - | 50 | 20 | 16 | 16 |
| 17c | - | - | - | - | 10 | 7 | - | - | - | - | - | 2 | 3 |
| 17d | 100 | 46 | - | 66 | 30 | 69 | 33 | 37 | 100 | - | 40 | 51 | 46 |

## 4. 1997 USAF LABORATORY HSAP MENTOR EVALUATION RESPONSES

Not enough evaluations received (5 total) from Mentors to do useful summary.

## 5. 1997 HSAP EVALUATION RESPONSES

The summarized results listed below are from the 113 HSAP evaluations received.

HSAP apprentices were asked to rate the following questions on a scale from 1 (below average) to 5 (above average)

1. Your influence on selection of topic/type of work.
2. Working relationship with mentor, other lab scientists.
3. Enhancement of your academic qualifications.
4. Technically challenging work.
5. Lab readiness for you: mentor, task, work plan, equipment.
6. Influence on your career.
7. Increased interest in math/science.
8. Lab research & administrative support.
9. Adequacy of RDL's Apprentice Handbook and administrative materials.
10. Responsiveness of RDL communications.
11. Overall payment procedures.
12. Overall assessment of SRP value to you.
13. Would you apply again next year? Yes (92 %)
14. Will you pursue future studies related to this research? Yes (68 %)
15. Was Tour length satisfactory? Yes (82 %)

| | Arnold | Brooks | Edwards | Eglin | Griffiss | Hanscom | Kirtland | Tyndall | WPAFB | Totals |
|---|---|---|---|---|---|---|---|---|---|---|
| # resp | 5 | 19 | 7 | 15 | 13 | 2 | 7 | 5 | 40 | 113 |
| 1 | 2.8 | 3.3 | 3.4 | 3.5 | 3.4 | 4.0 | 3.2 | 3.6 | 3.6 | 3.4 |
| 2 | 4.4 | 4.6 | 4.5 | 4.8 | 4.6 | 4.0 | 4.4 | 4.0 | 4.6 | 4.6 |
| 3 | 4.0 | 4.2 | 4.1 | 4.3 | 4.5 | 5.0 | 4.3 | 4.6 | 4.4 | 4.4 |
| 4 | 3.6 | 3.9 | 4.0 | 4.5 | 4.2 | 5.0 | 4.6 | 3.8 | 4.3 | 4.2 |
| 5 | 4.4 | 4.1 | 3.7 | 4.5 | 4.1 | 3.0 | 3.9 | 3.6 | 3.9 | 4.0 |
| 6 | 3.2 | 3.6 | 3.6 | 4.1 | 3.8 | 5.0 | 3.3 | 3.8 | 3.6 | 3.7 |
| 7 | 2.8 | 4.1 | 4.0 | 3.9 | 3.9 | 5.0 | 3.6 | 4.0 | 4.0 | 3.9 |
| 8 | 3.8 | 4.1 | 4.0 | 4.3 | 4.0 | 4.0 | 4.3 | 3.8 | 4.3 | 4.2 |
| 9 | 4.4 | 3.6 | 4.1 | 4.1 | 3.5 | 4.0 | 3.9 | 4.0 | 3.7 | 3.8 |
| 10 | 4.0 | 3.8 | 4.1 | 3.7 | 4.1 | 4.0 | 3.9 | 2.4 | 3.8 | 3.8 |
| 11 | 4.2 | 4.2 | 3.7 | 3.9 | 3.8 | 3.0 | 3.7 | 2.6 | 3.7 | 3.8 |
| 12 | 4.0 | 4.5 | 4.9 | 4.6 | 4.6 | 5.0 | 4.6 | 4.2 | 4.3 | 4.5 |
| Numbers below are percentages | | | | | | | | | | |
| 13 | 60% | 95% | 100% | 100% | 85% | 100% | 100% | 100% | 90% | 92% |
| 14 | 20% | 80% | 71% | 80% | 54% | 100% | 71% | 80% | 65% | 68% |
| 15 | 100% | 70% | 71% | 100% | 100% | 50% | 86% | 60% | 80% | 82% |

**Associate did not participate in the program.**

# AN INVESTIGATION OF THE PRECISION OF THE EL-MAR FIXATION ANALYSIS SOFTWARE TECHNOLOGY

Shannon J. Murphy

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119 E. Craig
San Antonio, TX 78212

Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
Boling Air Force Base, DC

and

Armstrong Laboratory

August 1997

# AN INVESTIGATION OF THE PRECISION OF THE EL-MAR FIXATION ANALYSIS SOFTWARE TECHNOLOGY

Shannon J. Murphy
Keystone School

## Abstract

The *el-mar* Fixation Analysis Software Technology(FAST) analyzes eye movements. Because FAST was built to support instructional applications, such as training novice pilots, the precision of FAST for detailed analysis has not been documented. I investigated to what extent the precision of FAST can be taken to be used in scientific research. The precision of FAST was investigated through a series of three data collection and analysis experiments performed on an eye scan instruction tape prerecorded with the Vision 2000 system. The first experiment was to determine the precision at which one file of collected data could be reanalyzed. The second experiment was to determine which system of restart data collection yielded the most precise numerical data. The third experiment was to determine the method of using the correct end field at which a specific length of the instruction tape could be recollected as data and analyzed with the most precision. Given the same data colection file and object table, experiment 1 proved that the actual analysis step of FAST will consistantly produce the same results (ie. 100% precision) and therefore any variability in the numerical data is due to data collection. The results from experiment 2 suggested that the variability in numerical data is not significantly effected by the restart system used in data collection. The results from experiment 3 suggested that the variability in numerical data continues with the minimization of human error. Therefore several replications of results are reccomented when performing a detailed analysis.

# AN INVESTIGATION OF THE PRECISION OF THE EL-MAR FIXATION ANALYSIS SOFTWARE TECHNOLOGY

Shannon J. Murphy

## Introduction

The *el-mar* eye-tracker Vision 2000 tracks the eye movements of a subject on his environment[2]. It's primary application has been as an instruction tool to teach novice pilots the correct eye scanning patterns in basic flight maneuvers[3]. The eye movements are presented as a real time visual scan superimposed on a video recording of the environment. Therefore the instructor can see precisely where and what object the subject's eye are fixating on at any time. The *el-mar* Fixation Analysis Software Technology(FAST) can be used to analyze the recorded eye movements to calculate the amount of time the subject spent fixating on any one object and how many times the subject fixated on the object in a given time period[1]. This kind of information could be very useful in scientific research. For example it could be used to test the effectiveness of new or improved flight instruments in increasing flight performance by allowing for more efficient eye scanning patterns to be used in basic flight maneuvers. Because FAST was built to support instructional applications, such as training novice pilots, the precision of FAST for detailed analysis has not been documented. I investigated to what extent the precision of FAST can be taken to be used in scientific research. In any analysis trial the investigator must follow a few general steps (see fig. 1). See appendix A for a detailed procedure for working FAST. See appendix B for a replication of the Main Form Window.

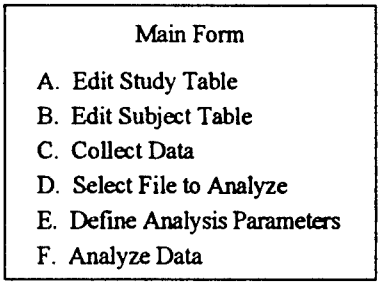



fig. 1

**Over View**

Methodology

The precision of FAST was investigated through a series of three data collection and analysis experiments performed on an eye scan instruction tape prerecorded with the Vision 2000 system. FAST presented numerical data only in steps C and F (see fig. 2 below) from Appendix A. Therefore steps C and F were the focus of this investigation to evaluate the precison of the numerical data.

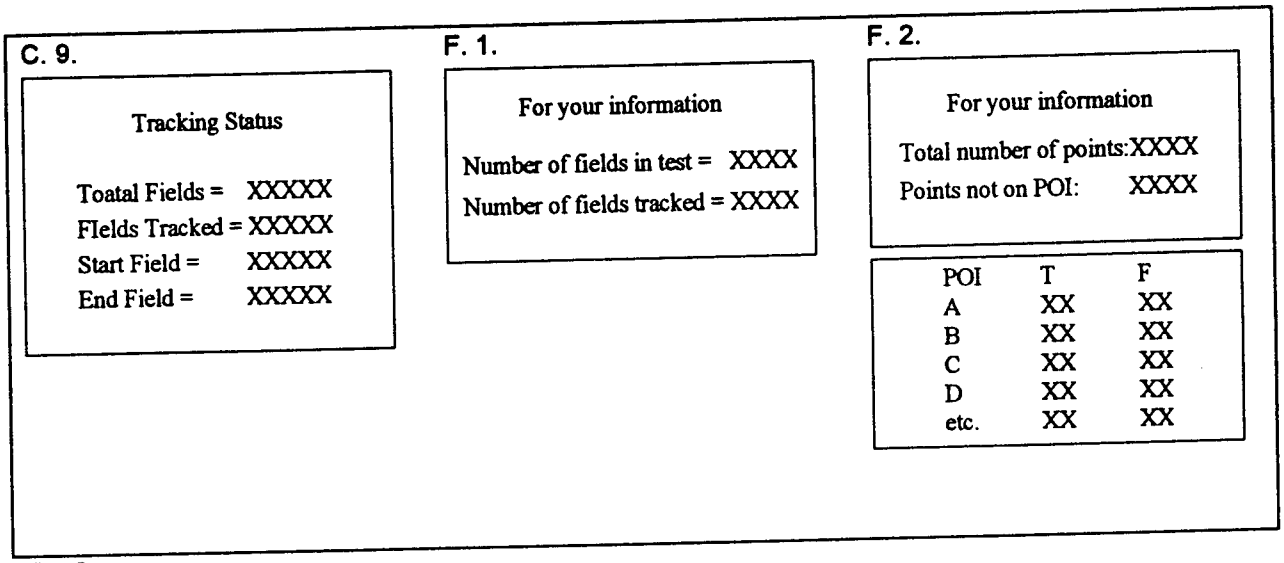



fig. 2

**Experiment 1**

Methodology

The first experiment was to determine the precision at which one file of collected data could be reanalyzed. To complete this experiment, the investigator performed step C once, producing a data file (MulAcc_1), and used the same object table from step E. The investigator repeated step F on the same collected data four separate times.

Results

The results of experiment 1 found that when reanalyzing the same data collection file witht the same anaysis parameters, the numerical data remains exactly the same creating a 100% confidence level. These results suggest FAST completes step F with 100% precision.

**Experiment 2**

Methodology

The second experiment was to determine which system of restart data collection yielded the most precise numerical data. The restart systems consisted of restarting the system all together by turning off the computer and resetting the eye-tracker(A), exiting the collect data menu and reentering (B), exiting out of paradox but staying in windows then reentering(C), and exiting out of windows to DOS then reentering(D). File names for this experiment were either AMuRes, BMuRes, CMuRes, or DMuRes depending on the restart system used. In one performance of step C the investigator collected data, stopped at the beginning of a particular second, recollected, and tried to stop at the exact same spot until 10 end field points had been collected. This procedure was repeated 8 times each for 4 different restart systems except for restart system C which was repeted only four times.

Results

The data showed no significant difference in the level of precision with each of the four different restart systems. The results in fig 3 show that the precision with which an investigator can stop on a specific frame is within $\pm$ 10 frames for n = 280. One possible explanation for this pattern is based on the fact that there are 60 frames that can be tracked in one second so that even if the investigator stopped data collection in the same second, the frame number could still vary. Therefore restart system B was selected for use in further experiments because it was the most time efficient.

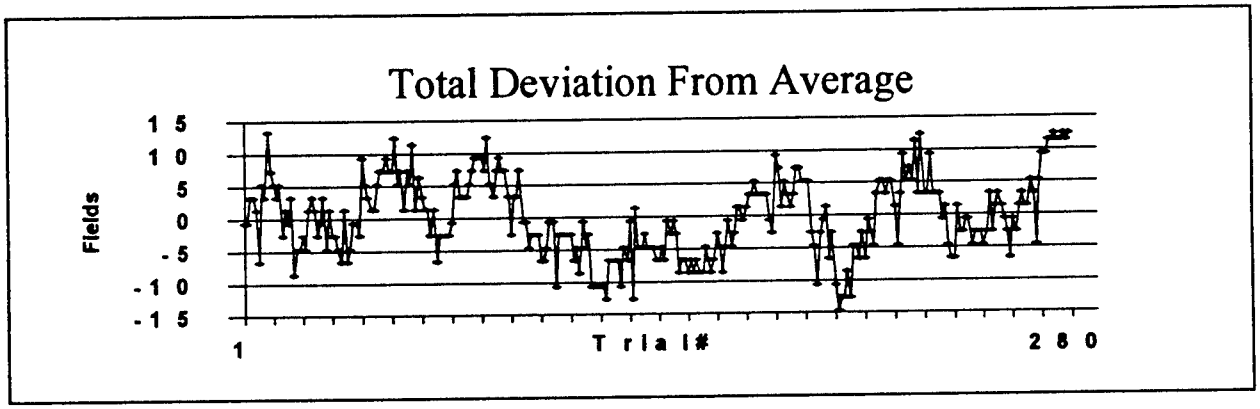



fig. 3

**Experiment 3**

Methodology

The third experiment was to determine the method of using the correct end field at which a specific length of the instruction tape could be recollected as data and analyzed with the most precision. In this experiment the investigator collected data(step C) in two mehtods for acquiting the end field (step C9). For the first method, the investigator stopped at the beginning of the predetermined end second (eg 6:33) of the task. The end field acquired was the end field entered in step E. This method of data collection was labeled A (file name: A__Tes). The investigator completed these steps nine times by method A, each with the same number of total fields but with varying end fields from step C. The second way the investigator preformed step C was to stop data collection past the end of the task. The average of the end fields collected by method A was then entered as the end field entered in step E. This method of data collection was labeled B (file name: B__Tes). The investigator completed these steps nine times by method B, each with not only the same total fields, but the same end and start field. Both methods were analyzed (step F) using the same object table from step E.

Results

The results of the third experiment showed no significant difference in the precision of data collected through method A or method B. Figure 4 shows graphs of time and fixations (numerical data from step F2) by object for mehtod A and B. Figure 5 shows graphs of the total time and fixations by trial for method A and B. The total seconds per trial was about 80.92 sec with a standard deviation of $\pm$ 1.405 sec for n = 18 at a confidence level of 95%. The total fixations per trial was about 166.7 fixations with a standard deviation of $\pm$ 1.2 fixations for n = 18 at a confidence level of 95%. Most deviations in time and fixation data coincided with the fact that the number of fields tracked was less than the total number of fields in the test. Focusing on the numerical data presented in step F1, on average only about 94.9% of the fields were tracked with a standard deviation of $\pm$ 1.2 % for n = 18 at a confidence level of 95%. The reason why the time and fixations vary for each trial is is not only because not all field were tracked, but

because each data collection it is not always made up of the same points that make up the fields that were tracked. Therefore, even if the same percentage of fields were tracked for every trial, the numerical data recorded in step F2 could still very.

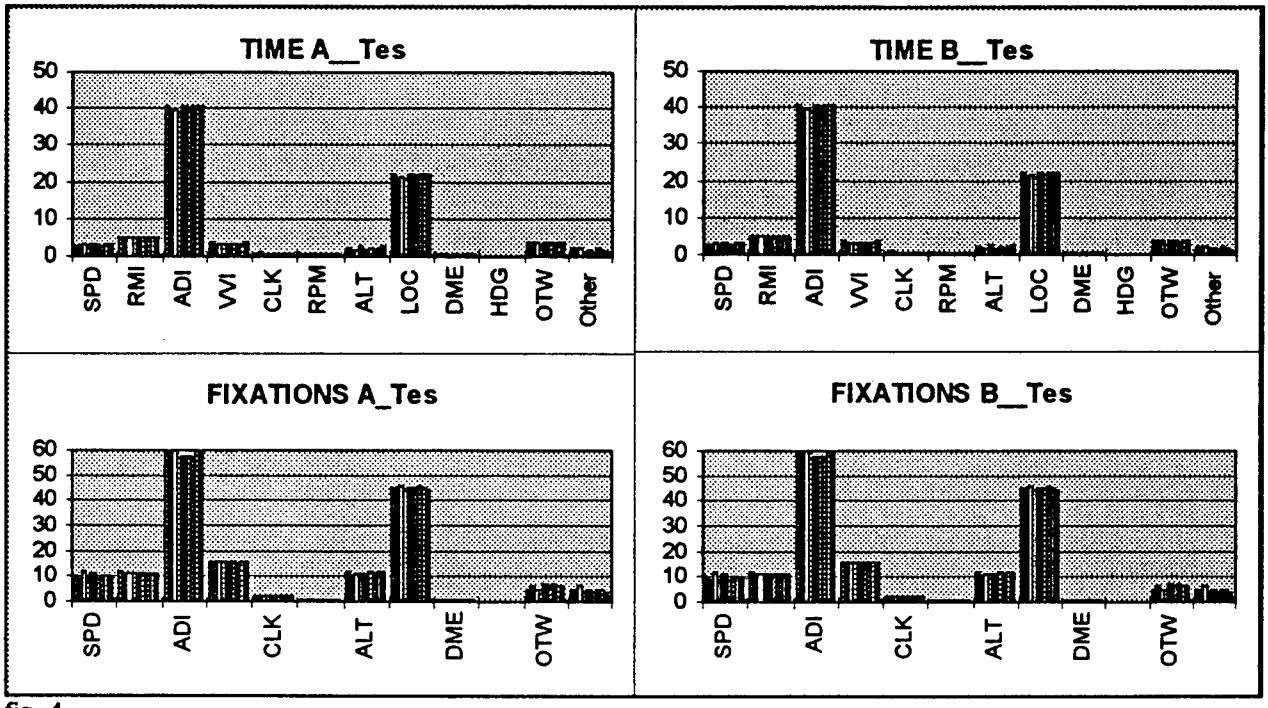


fig. 4

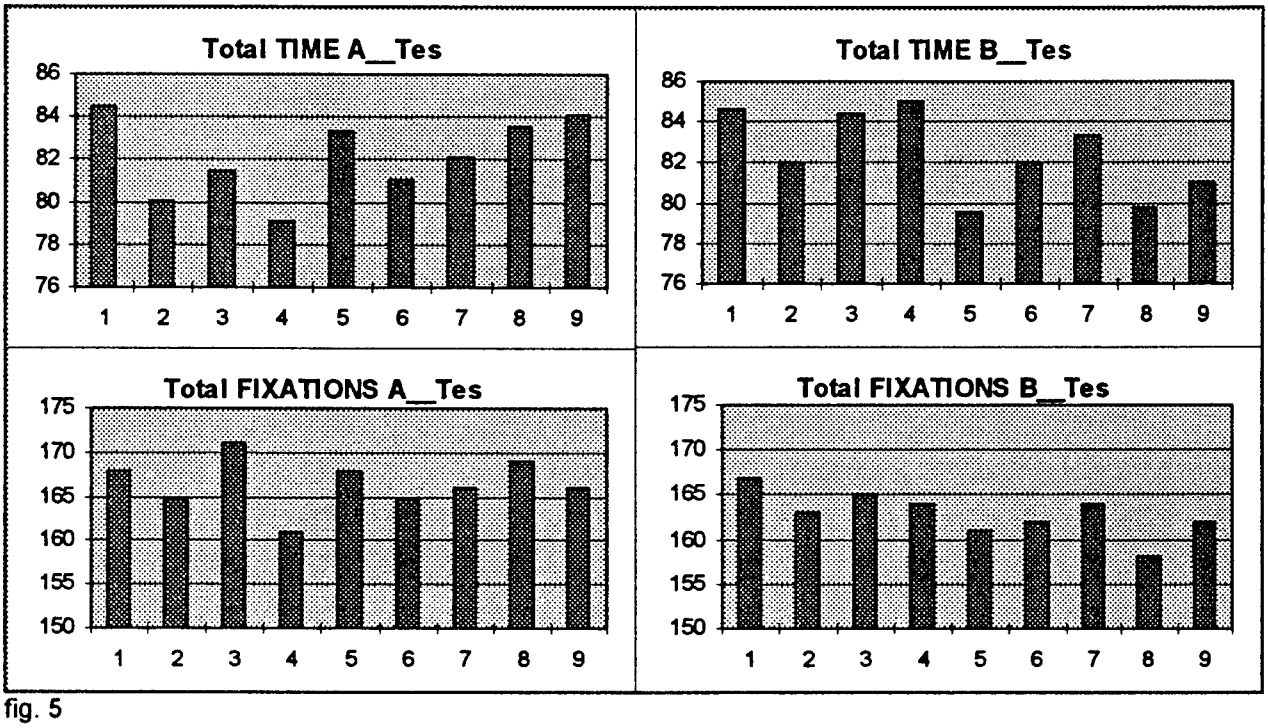


fig. 5

Conclusion

Given the same data colection file and object table, experiment 1 proved that step F will consistantly produce the same results (ie. 100% precision) and therefore any variability in the numerical data is due to step C. The results from experiment 2 suggest that the variability in numerical data is not significantly effected by the restart system used in step C. The results from experiment 3 suggest that the variability in numerical data continues with the minimization of human error. However, the data supports the possibility that FAST's inability to track all of the fields in a test durring step C caused the lack of precision in the data. Therefore several replications of results are reccomented when performing a detailed analysis.

Suggestions For Future Investigation

I have noticed in my investigation that the percent fields tracked in a test depends on the quality of picture captured to define the targets in step C3. I predict that an increase in the quality of target defining picture will increase the percent fields tracked in a test and therefore maximize the precision of FAST.

Appendix A

## Detailed Instructions for the El-Mar Eye-Tracker Analysis System

### I. SET-UP

**FAST** - enter windows, auto analysis window, paradox for windows(Printer Error OK), main form

**Vision 2000**

1. make sure the Analyze/Run switch is switched to Analyze
2. turn on power to VCR, rewind tape and counter reset

### II. ANALYSIS

**Computer Main Form**

A. Edit Study Table
   1. enter new test # and name and close form
   2. scroll down to correct test and select

B. Edit Subject Table
   1. modify subject table Yes
   2. enter subject #, last & first name, sex, age, & date in dd/mm/yy form then close form
   3. scroll down to correct subject and select

C. Collect Data
   1. use tape keys on VCR or monitor to get tape to where you want to start tracking
   2. click play(triangle) then video recorder picture on the monitor
   3. click the camera picture to take a snap shot frame (click the camera as many times as necessary to get a suitable snap shot)
   4. click the automatic light adjuster button then click on the white center of a target (if it works click yes, if not try again on another target)
   5. click the target button then on the center of all visible targets (only need two but more is better)
   6. make sure you set the VCR tape within 10 sec from where you want to begin tracking
   7. click Begin Tracking
   8. click the right mouse button to end tracking
   9. record by hand Total fields, Fields tracked, Start field, & End field
   10. exit to main form

D. Select File to Analyze - select the name of the file just analyzed

E. Define Analysis Parameters
   1. enter test name etc. and the start and end field with in the start and end fields collected
   2. save test table

3. Get Test Images
    - click eye button
    - Get Test
    - click on right mouse button to end search
    - manually choose own test image Yes
    - take snap shot and define target just like in Data Collection steps 2-5
    - save image
    - exit back to Define Analysis Parameters menu
4. Select test
5. enter # and name for all objects to be defined on the table
6. click Get Position and drag boxes around the objects entered in object table then click Finished
7. adjust object boxes by manipulating the numbers under Xpos, Ypos, Dx, & Dy on object table
8. click away from most recently entered cell and click View Object to view changes
9. Save Object List
10. Return to previous form

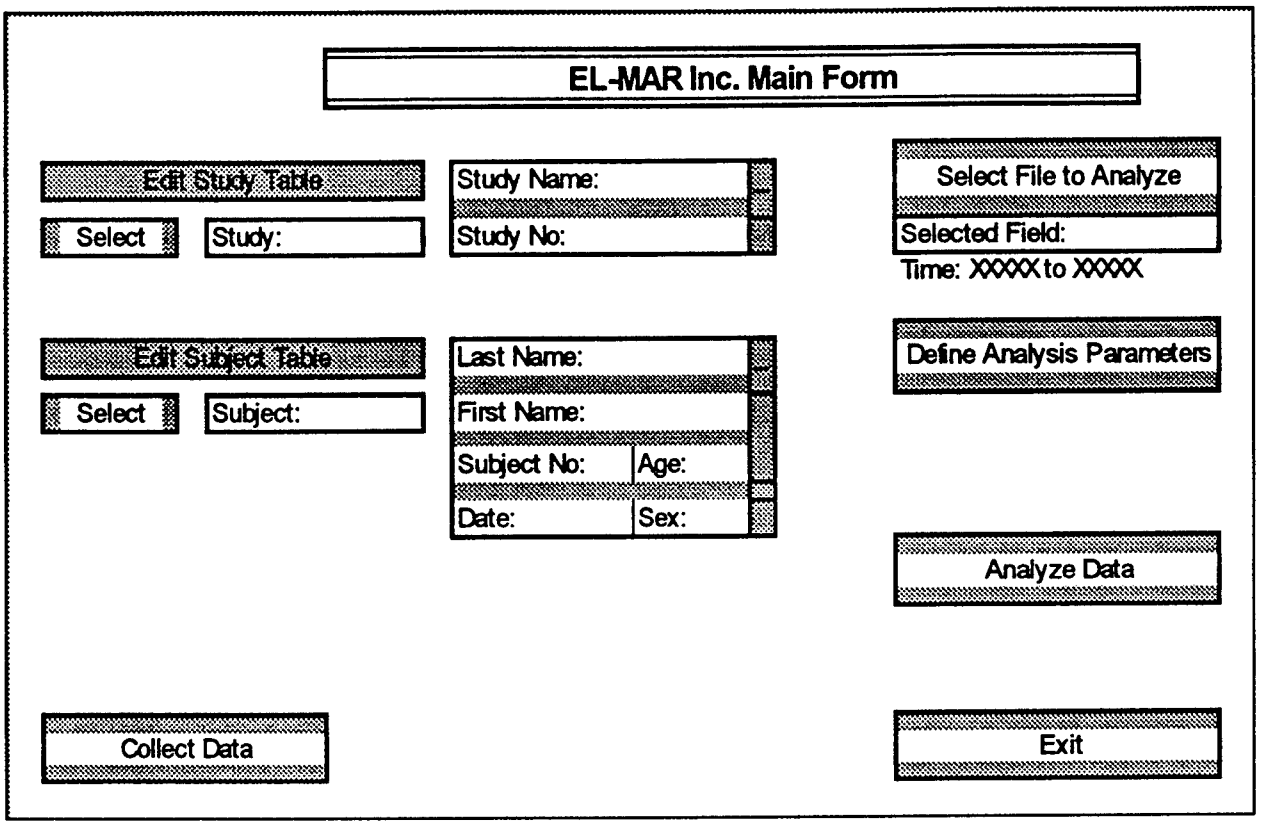
F. Analyze Data - current subject
1. Get Data - selected test
    - record manually # Fields in Test & # Fields Tracked
    - click OK
2. Analyze Data - pictorial form
    - record Total # Points & Points not on POI
    - click OK
    - record time & fixations for object list then click Finished
3. Return to previous form

When collecting multiple files on the same subject

1. Collect Data
2. Do you want to over right this file? No

When using the same test, subject, object list, or object picture, those parts of the procedure can be skipped.

Appendix B


EL-MAR Inc. Main Form
Edit Study Table
Select
Study:
Study Name:
Study No:
Select File to Analyze
Selected Field:
Time: XXXXX to XXXXX
Edit Subject Table
Select
Subject:
Last Name:
First Name:
Subject No:
Age:
Date:
Sex:
Define Analysis Parameters
Analyze Data
Collect Data
Exit

## References

[1] "Fixation Analysis Software Technology" Version 2.03, EL-MAR Inc. 1997.

[2] "Vision 2000 Video Eye-Tracking System" Version 1.0, EL-MAR Inc. 1995.

[3] Wetzel, Paul A. and Kruger-Anderson, Gretchen. "An Eye System for Analysis of Pilots' Scan Paths".

# METALS ANALYSIS BY ATOMIC ABSORPTION USING A GRAPHITE FURNACE

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

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Armstrong Laboratory
Brooks AFB, TX

August 1997

# METALS ANALYSIS BY ATOMIC ABSORPTION USING A GRAPHITE FURNACE

Katrina Navalta
Northside Health Careers High School

## Abstract

Atomic absorption spectrophotometry has become a method for chemical analysis of trace metals at the parts per billion level in solution. It is a useful and popular technique because it can be applied to many different fields of science: agriculture, biochemistry, the environment, food products, forensic chemistry, geochemistry, industrial analyses, metallurgy, petrochemistry, pharmaceuticals, cosmetics, plastics, and fibers. The modern technology of the graphite furnace dates from about 1980 yet it has developed with tremendous speed into becoming the most widely used method for determining metals at ultratrace levels in biological materials. The Perkin-Elmer 5100 PC Furnace Atomic Absorption Spectrophotometer is used to analyze metal elements in air and bulk samples with very low detection limits and small volumes. This is a machine with great versatility and is fully automated. The metals analyzed by this instrument are: arsenic, antimony, cadmium, lead, selenium, thallium, and tin. Before analysis, there is a certain way to digest each type of sample received. Each includes the addition of nitric acid to break down the sample and concentrate it into an assigned final volume. This concentrates the sample so the condensed volume will be free of solids and the whole sample can be analyzed at an amount of 20 microliters but still representing the whole sample. Many considerations are made before each run, including parameters for the analyte and background interferences, but overall, this is a field on analytical chemistry that will continue to develop into faster and more convenient ways for the detection of metal elements in solutions. I digested a lot of samples (ex. dirts, paint chips, sludges, gauze swipes, air filters, hazardous waste) in a laboratory setting over the hot plate or through microwave digestion. There was no experimental research involved, we had set guidelines to follow for digestion and operation of the instruments for an analysis of a sample.

# METALS ANALYSIS BY ATOMIC ABSORPTION USING A GRAPHITE FURNACE

Katrina Navalta

## Introduction

"Atomic absorption is the process that occurs when a ground state atom absorbs energy in the form of lights of a specific wavelength and is elevated to an excited state" (Perkin-Elmer). An atom of an element is surrounded by a unique number of electrons. When an atom is given a certain amount of energy the energy is absorbed and one of it's outer electrons will become excited. A decay process will occur and the electron will return to the initial, stable orbital position. A characteristic wavelength of light can be uniquely related to a certain element. Energy is released in the form of light and provides the key factor for identifying and measuring amounts of an element in a sample. Measurements of the amount of energy absorbed or emitted by this excitation/decay process is used for analytical purposes. Light emitted during the decay process can be used to identify an element. Each element has a unique electronic configuration and emits and absorbs light energy at specific wavelengths. Unknown concentrations can be determined by comparing the measurement of the amount of light they absorbed to the measurement of the amount of light and the concentration of the analyte in known concentrations. These concentrations are electronically calibrated by the instrument. A primary light source, an atom source, a monochromator, a detector, electronics, and a date display or logging device are the basic instrumentation for atomic absorption (AA). The intensity of light emitted during the decay process can measure how much of an element is a sample being analyzed. Atomic absorption spectrophotometry (AAS) is when an atom can absorb specific wavelengths of light. This is why an element can be tested in the presence of others using AAS, certain lamps and specific wavelengths can be programmed to run the analyses of a graphite furnace. The science of AAS in combination with the graphite furnace uses the calculations of light emission and absorbency of an excited atom in a sample to find its quantity or find out what elements are present in a sample sent to the laboratory for analysis.

The samples that the Perkin-Elmer 5100 PC Furnace Atomic Absorption Spectrophotometer receives for analysis include air samples collected on mixed cellulose ester filters, sludges, soils, paint chips, dirt, and effluents

from industrial waste treatment facilities (Analytical Services). Blood containing lead, and drinking water containing lead and copper may also be analyzed by the graphite furnace. The sensitivity of GFAA is an advantage when a sample comes into the laboratory with a very low concentration. The graphite furnace enables testing in very small quantities, microliters, and testing to a sample's parts per billion factor. Using GFAA, certain types of solid samples can be analyzed directly. Sample throughput for this method is based on single element analysis. Therefore, the number of samples that can be analyzed or elements which can be determined per unit time is very low. It usually takes two to three minutes for determination of a sample because before the atomization step in this technique, the solvent and matrix components need to be removed. The graphite furnace operates with autosamplers. They mechanically introduce the sample into the graphite tube onto the L'vov platform. They can also make up standards on its own and set modifier into the furnace so the sample does not heat up too quickly. Their advantages are automation and improved performance. It accurately measures amounts of fluid and operates at specific times, which leaves the sample preparation process with less chance of human error. Microliter sample volumes are analyzed by the graphite furnace. Over forty elements may be used in the GFAA technique, including arsenic, antimony, cadmium, lead, selenium, thallium, and tin. Solid samples may also be introduced into the graphite furnace by different means, which has some advantages. This method requires little or no sample preparation, reduces total analysis time, minimizes the potential for contamination or analyte loss, and reduces the cost of sample pretreatment regents and their disposal (Beaty, 5-14). Slurry sampling is when the sample is made into a very fine powder. This is accomplished through freeze-drying or grinding. This analyte is made into a solution by adding liquid. Graphite furnace atomic absorption is a method for finding ultratrace metals in solutions. The samples received into the laboratory are digested according to type and then introduced into the pyrolytic-coated graphite tube of the furnace for analysis as a concentrated solution.

Before a sample is introduced into an instrument for analysis, they are prepared so they become solids free and are concentrated into a solution so that if any measured amount of it were to be extracted for analysis in the laboratory, it would represent the whole volume of sample. Air samples collected on mixed cellulose ester filters may be digested over the hot plate or in the multi-wave microwave digestion system. It depends on whether the elements contained on the filters are ones that may be lost in the extreme temperature of the hot plate, if so, it is

digested by microwaves. Procedure for digestions taking place on the hot plate, use three mLs of ultra-pure nitric acid to break down the filters. By adding one mL of 30 percent hydrogen peroxide (in two separate additions of 0.5 mL), it acts as a catalyst in the chemical reaction and speeds up the process of further breaking down the filter so that all elements in the filter are put into solution. The solution is placed in a fifteen mL tube and double-deionized water is used to fill the remainder of the centrifuge tube to fifteen mL, since what is digested comes out to a total volume of about ten mL only. Microwave digestion is different. Teflon vessels and caps are used to hold the filters in a tray for the microwave. Three mL of ultra-pure nitric acid is added and one mL of 30 percent hydrogen peroxide accompanies the filter for digestion in the microwave for approximately forty minutes. These are also prepared for a fifteen mL tubed solution. As for paint chips, about 0.1 g of sample is weighed and 15 mL of ultrapure nitric acid is used for digestion. Again, 1 mL of 30 percent hydrogen peroxide in the digesting process acts as a catalyst. The paint chips break down quite easily and are dissolved into a solution which will be prepared for a fifteen mL tube also. Ribbed and plain watchglasses are used for hot plate digestions because this prevents spattering of sample from one beaker to another and it also condenses the sample. When the solution cooking on the beaker vaporizes it hits the watchglass and droplets of samples remain in the beaker that way. The digestion procedures for the remaining types of samples that may be introduced into the graphite furnace include sludges, soils, and dirt and effluents from industrial waste treatment facilities. These digestion procedures are very similar to the digestion of paint chips with exception to the amount of nitric acid and hydrogen peroxide used. They need a longer digestion period and require the use of glass boiling beads so the effervescence of the hot sample doesn't overflow the volume of the beaker.

Discussion of Technique

Graphite Furnace Atomic Absorption (GFAA) is a very sensitive and improved sampling device. it is based on the process of atomic absorption spectrophotometry and uses light emission and the amount of light absorbed in a cloud of atoms for analyses of metallic elements. The atom source produces free analyte atoms from the sample which help to indicate the quantity of an element. "The source of energy for free atom production is heat, most commonly in the form of an air-acetylene" (Perkin-Elmer, 2). Beer's Law is the relationship of light absorption with concentration. It is: A=abc. Absorbance is equal to the absorption coefficient multiplied by the

length of the light path multiplied by the concentration of an absorbing species. The absorption coefficient is the common value of the absorbing species at a specific wavelength. The light path is the one intersecting with the absorption species in the absorption cell. This is helpful when calibrating the system to work with a specific element because it uses the relationship of it's characteristic absorbance with the concentration of the absorbing species for a given set of instrumental conditions. Therefore, the calibration curve may be used to find any unknown concentrations by measuring the absorbance of solutions and by comparing the results to the standards that contain known concentrations of an analyte. When the sample is introduced into the tube, it is heated. It can hold the atomized sample in the light path for an extended period of time. Inside the pyro-coated graphite tube is the L'vov platform. It is a small plate of solid pyrolytic graphite that hold the sample solution when it is in the atomization step. As atoms are formed and diffuse out of the tube, the absorbance rises and falls in a peak-shaped signal. The peak height or integrated peak area is used as the analytical signal for quantitation. This addition of the L'vov platform into atomic absorption instrumentation allows the vaporization of the sample into a higher temperature gas atmosphere producing more free atoms and it also produces less interferences. It prolongs the tube life, too, because hostile samples that may do damage to the graphite tube will be in contact with the platform only. It may hold up 50 uL of sample.

According to Perkin-Elmer, "Graphite furnace atomic absorption (GFAA) allows the determination of over 40 elements in microliter sample volumes with detection limits typically 100 to 1000 times better than those of flame atomic absorption." When determining an analytical technique, take into consideration: detection limits, analytical working range, sample throughput, cost, and interferences. In this technique, a tube of graphite is located in the sample compartment of the AA spectrometer, with the light path passing through it. A small volume of sample solution is quantitatively placed into the tube, normally through a sample injection hole located in the center of the tube wall. The tube is heated through a programmed temperature sequence until finally the analyte present in the sample is dissociated into atoms and atomic absorption occurs. The typical detection limits for the graphite furnace range from 0.1 to 0.01 ug/L. This signifies how well a particular element will be analyzed by the instrument. An analytical working range is important because it enables you to analyze samples with different concentrations at one time. Thus, an analytical working range can be defined as the concentration range over how

many different samples it can analyze without recalibration. When analyzing through a graphite furnace, usually a single element is being tested from each sample. The sample throughput for the furnace is low, meaning the number of different elements it can analyze at one time is only one at a rate of 2-3 minutes per sample. Volatile and medium refractory elements are recommended for analysis using this L'vov platform. Although the method for analyzing elements by the graphite furnace atomic absorption method is very practical there are also some major interferences. Matrix interference, chemical interference, ionization interference, and background absorption need to be taken into consideration. These can be corrected by using sample preparation caution, method of standard additions, an ionization buffer, and an instrumental correction technique such as continuum or Zeeman background correction. Background absorbance is an interference effect due to spurious radiation absorbed at the same wavelength as the element of interest. You will know whether these interferences are effecting your analyses with the graphite furnace if you cannot calibrate the system right or your standards are not calculating out to the known concentration.

Methodology

In general, samples are prepared such that the resulting solution are free of particulate matter and has a matrix of two to three percent ultra-pure nitric acid. All aqueous standards, a minimum of three, will also be prepared in three percent ultra-pure nitric acid. These will be used to calibrate the instrument and prepare it for analyzing the elemental sample. The autosampler can create working standards from stock standard solutions, add appropriate reagents, and provide method of additions analyses or recovery measurements, all automatically. The practical quantitation limit (PQL) and the linear working range of the sample being tested are what determines how a sample is to be prepared by the analyte. According to Analytical Services: A run spike will be prepared by diluting 50/50 a blank air sample with the highest calibrator. A sample spike (matrix) will be prepared at the time the samples are being digested. In general, no dilutions will be made more than one hundred fold at one time and the smallest volume of concentrate will not be less than 100 microliters. The graphite furnace's signals depend on analyte mass. An analyst can control sample volume and also control measured absorbance by understanding that these two elements are related. In a sample solution with a large volume there is more analyte present to be tested so the signals picked up by the monochromator are greater. By varying sample

volume, the analytical range of the analysis can be controlled.

A previously saved method, element and multielement methods, can be retrieved from data stored on a disk. At Armstrong Laboratory there are certain specifications, and there is a certain procedure that is followed, in order to operate the graphite furnace. The following instructions are taken from the Analytical Services SOP 48-908:

1. Power: Turn the main instrument on by depressing the ON key on the ONEAC voltage stabilizer. Turn on the cooling system. It will take about two minutes for the instrument to go through its diagnostics.

2. Gas: Rotate the gas cylinder valve counter-clockwise for 4-5 turns. Ensure that there is at least 60 psi in the argon tank.

AUTOMATED ANALYSIS SET-UP

3. When the AA Winlab software window appears, click on the Automated Analysis button.

4. While on the automated analysis page, double click on the Untitled menu and select the element to be analyzed.

5. Click on the Sample Information file and select the appropriate metal.

6. Click on the Use Entire Sample Info file.

7. To set up a work page, click on the Work Space icon and select Ken 1.

8. To set up the sample information file, click the Sample Info icon.

9. In this file enter the file name, run number, and the sample numbers to be analyzed. Save the file and close it.

10. When the automated analysis page re-appears, click on analyze located on the bottom of the page.

Measuring and dispensing a known volume of sample into the furnace are two main steps in the analysis of a sample using a graphite furnace. Using an Atomic Absorption Laboratory Benchtop system, a multi-step temperature program is activated. One advantage of using this program in accordance with the furnace is that it leaves the analyst with time to do other duties. It is fully automated and it can perform the remainder of the process by itself until the analyst must form a run packet for submission. "The Model 5100 PC is completely controlled from a personal computer with Model 5100 PC operating software. Multiple functions can be displayed on the screen so that all aspects of an analysis can be monitored at one time." (Perkin-Elmer) The atomic absorption measurement is made when the temperature in the furnace reaches a point where the analyte atomizes.

Heating parameters for each step that are vital to receiving accurate data include: final temperature during step, time for temperature increase, time for maintaining final temperature, and gas type and flow rate. The spectrometer can also be adjusted to an analyst's specific choice, too. This can be accomplished by modifying the graphite furnace program. This program includes drying, pyrolysis, cool down (optional), atomization, clean out, and cool down. The steps from the Analytical Service SOP 48-908 direct the analyst to:

1. To set the sample tray, place the samples in the appropriate position according the sample info file. Water is to be put into position 36, the standard in position 37, the matrix modifier in position 38, the mid-level standard check in position 39, and the quality control in position 40.
2. Click on the Calibrate button and allow the instrument to perform a calibration curve. If the correlation coefficient is greater than 0.995, the instrument will allow the analysis to continue. If it is not, the instrument will perform another calibration. If the QC is still not greater than 0.995, the instrument will stop.
3. Click on the Analyze Samples button to begin the analysis. The mid-level and QC are the first two results obtained. If they are within range, allow the analysis to continue. If they are not within the range, stop the analysis and determine what has gone wrong. Once the mid-level and QC are correct, proceed with the analysis.
4. After all the samples have been analyzed, click on file, then print sample information.

In preparation for analysis, you must define certain parameters and procedures for the type of sample being tested. Methods for detecting metal elements in air and bulk samples, using the graphite furnace, may be stored in the computer and need little modification when performing certain analyses. A method check list includes the following: Plans for preparing blanks, calibration standards, and samples. An element file for each element you plan to determine. A multielement file that links element files to automatically determine two or more elements in a given sample in one sample run. A list of QC samples, if required, to be analyzed at various points in the analytical sequence. A list of autosampler locations where the different solutions should be placed. The extent of method development is measured by the frequency of analyses and the content of the samples. Batches of samples with similar content and ones that will be tested frequently should have element files already stored in the computer. The graphite furnace is the best atomic absorption technique for analyses using samples with very low concentrations or small volumes. To better stabilize the sensitive environment of the graphite furnace, the graphite

tube and all graphite parts--contacts, tubes, and platforms--are pyrolytic-coated. "Mechanical dimensions of the graphite tube must be controlled within very narrow tolerances." (Perkin Elmer). In the Analytical Service SOP 48-908, it instructs:

1. There are up to eight different quality control checks during each analytical run. They are a blank, a QC, two mid-level standard checks, a run duplicate and run spike, a sample duplicate, and a matrix spike. The PQL standard is now part of the calibration curve.
2. Quality control charts are maintained in real time for all eight QC checks.
3. After a successful calibration has been established, perform a blank analysis. The blank result should be less than the PQL by a factor of five. If it is not, change the wash water in the basin, put a new blank in the carousel and analyze again. If it is still too high, the graphite tube will probably need replacing.

Quality control check ensure that the instrument is working efficiently and is returning data that is accurate and that will give the customer true results from the analysis of their sample(s). It is an important factor in determining the quality of the laboratory and their analysts as well.

Results

After completing a run, analysts are required to turn in all data from the analysis along with an occupational metals checklist to someone for technical review. This packet of information is signed and checked by the supervisor. On the occupational metals checklist you indicate what element(s) you analyzed in the column listed under your instrument. Include the run number, date, and your signature. This checklist also requires you to initialize 5 tasks to be sure that you completed them: standards logged into prep log, PM/FV log completed, all QC checks within specification (QC Summary Sheet), QC charts up-dated and submitted with run (SPCEX), sample results annotated on worksheets. Calculated results are taken from the computer generated date of the analyses. These should have correct units, method numbers, run numbers, and initials. The final run packet includes the computer generated run list, the hand-written run list, the analytical run, and the QC summary sheet. The SPC QC contains all of the previous analyses' QC summary sheets. If there is an out-of-control point or a trend in the graphs generated from the data entered of the QC summary sheets they will be immediately recognized by the viewer. Before any of the results can be released, these problems have to be addressed and resolved. "The

Function Chief will send the completed worksheets to Technical Services for generation of a final report. When the report is finished, the Chief will marry the Form 2750/51 with the report and perform an administrative review. He will then sign the final report cover page and present the packet to the Branch Chief for final review" (Analytical Services).

Conclusions

The results generated by the Perkin-Elmer 5100 PC Furnace Atomic Absorption Spectrophotometer provide information for the many people that are concerned with various environmental hazards. For example, in 1971 steps were taken to reduce the use of lead-based paint. The Lead-Based Poisoning Prevention Act (LBPPA) led to the Consumer Products Safety Act (CPSA), which restricts the amount of lead in paints manufactured after February 27, 1978. By analyzing paint samples for percentages of lead contained, someone can be saved the risk of lead poisoning in the workplace, home, or recreation area. The analysis of lead in blood can prevent cases of childhood lead poisoning from occurring. "Childhood lead poisoning is a major public health problem in many industrialized countries" (Parsons, 925). The National Lead Laboratory Accreditation Program (NLLAP) is another organization that uses these results for the benefit of others in danger. In one case, Lackland Air Force Base in San Antonio was able to satisfy The Resource Conservation and Recovery Act (RCRA), which keeps track of hazardous waste until it's final disposal. Many samples are received by the laboratory under this act and data is relayed back to the customer as soon as possible. People who live in areas with very high traces of metals in their drinking water are concerned for their health and send samples of water to laboratories to have them tested. The U.S. Environmental Protection Agency (EPA) is responsible for making sure that drinking water is safe for Americans to drink. They send samples from aquifers and drinking water sources to laboratories to have them tested for to make sure they pass health regulations. The results are used to indicate whether a person's atmosphere is in accordance with mandated living conditions. Samples can also be taken of river sediment. These can be tested to see if an industry down the river has contaminated the surrounding environment. The results of this can be a thorough clean-up of the area and residents can be saved from the harmful effects of exposure to excessive lead and other elements in their system.

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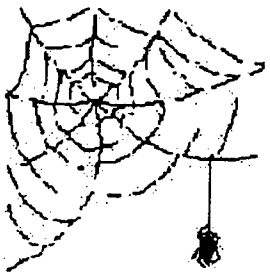

# SPINNING A WEB

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Final Report for:
High School Apprenticeship Program
Armstrong Laboratory
Brooks Air Force Base

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# SPINNING A WEB

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## Abstract

With over 30 million Web pages online and more being launched each day, Internet users can easily be overwhelmed by the tremendous amount of information available. To entice and maintain an audience, institutions must make sure their Web sites are navigable, useful, and attractive. This means that before creating any Web documents, developers must outline goals and formulate a general information architecture. A well structured content is instrumental in producing a successful Web site. HTML coding of the first draft design should be followed by several rounds of design reviews and revisions. In addition, user testing enables the identification of any potentially confusing areas. Only after the material has been fully developed and tested should the Web site be made available to the general public. This paper documents how this product development cycle was used to create a Web site for the Armstrong Laboratory Cognitive Task Analysis group.

# SPINNING A WEB

Christine P. Pan

## Introduction

Over the last several years, the World Wide Web has grown to become an integral form of communication for millions of people. Accessing and providing digital information is now easy and inexpensive with this potent technology. People are noticing the tremendous potential for this universal information database. Establishments everywhere, eager to take part in this new electronic world, are setting up their own web pages. Just by making their information available on the Internet, institutions develop a Web presence that could be the catalyst for higher levels of success. The reason is a Web site greatly increases customer base. It is an excellent way for businesses, government, consumers, and academia to interact.

The Armstrong Laboratory Cognitive Task Analysis (CTA) research group wanted to develop such a Web site that researchers and others could visit to obtain information about the CTA research being conducted by the Air Force. However, quickly designing and developing a navigable, attractive, high-quality site that could be easily expanded is a daunting challenge. This paper documents the steps I took to develop a Web site for the CTA team.

## Background

The word "Internet" is used to describe the global network of computers. Sometimes called "the information superhighway," "cyberspace," or "the net," it commonly supports services like electronic mail, the World Wide Web, and electronic file transfer.

The World Wide Web refers to a body of information. It was created by Tim Berners-Lee in March of 1989 while working at CERN, the European Particle Physics Laboratory (Zeltser, 1995). He wanted people all over the world to be able to share information by putting it on a web consisting of hypertext documents. Internet users immediately embraced the Web.

Hypertext, a word coined by Ted Nelson in 1965, describes text that includes

links or shortcuts to other documents, thus providing users an alternative to page-by-page exploration. HyperText Markup Language, or HTML, is the language used to construct Web pages, which in return, make up the World Wide Web. HTML can be written in almost any plain text editor.

Methodology/Results

Creating an effective commercial Web site requires planning and effort, just like any other aspect of business development. My first step was to determine the needs and goals of the CTA team. They wanted to make information available about the Air Force CTA work available over the Internet. At the same time, the CTA team wanted to create a relaxed, friendly atmosphere for the viewer. After brainstorming, the CTA team and I developed a list of information and services we wanted to offer on our Web site.

To improve my proficiency in writing HTML, I read two books: Using HTML, 2nd edition by Tom Savola et al., and The HTML Sourcebook, 2nd edition, by Ian S. Grahm. I utilized the Internet to look at many on-line tutorials. As an exercise, I spent some time exploring Web sites and their associated home pages, comparing the different designs and layouts. In addition, I took notes on how to code for different formats, such as tables, outlines, forms, and lists. Plus, I discovered how to insert audio clips, create different colored links, and adjust the size of images. Along the way, I created a Web page (as shown in Figure 1) using the new techniques I learned. The Web page was originally in outline format, but as my skills improved I converted it into a table. After I became fairly comfortable with writing HTML, I began to work on the CTA team Web collection. I browsed numerous Web sites, gathering animated gifs, backgrounds, rulers, and icons to use on the page.

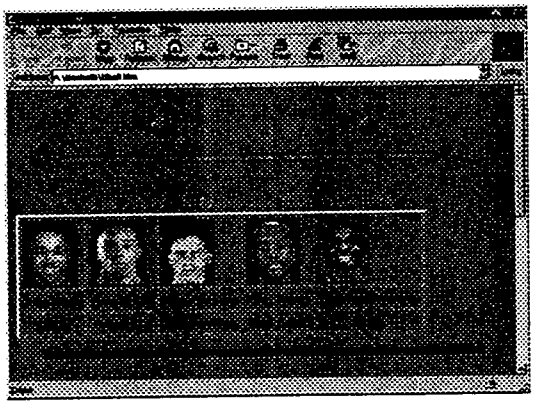

Figure 1. Practice Web page, with table incorporated.

One of the most important things to consider in designing a Web site is its structure. A sturdy information architecture is crucial in making a Web site expandable, memorable, and user-friendly. However, consideration should also be given to the screen design. Since all information on the Web site is presented visually, the format of the content displayed impacts the efficiency of the interaction between the user and the computer. In a study, Keister and Gallaway discovered that redesigning a series of screens resulted in a 25% reduction in total processing time and a 25% reduction in error rates (cited in Helander, 1991).

With that in mind, I planned the framework using an organizational chart. The home page would have an introduction and a table consisting of links to the major divisions that were defined with the help of the list we created during the group brainstorming session. Along with names for those divisions, I made up epithets further describing the content users will find under each one.

Once the planning was done, I began coding. Using my organizational chart as a guide, I wrote the first draft (see Figure 2). Each page had a hypertext menu enabling readers to easily skip to different sections. Since users are often left wondering how current a particular piece of information is on the Internet, I made sure to include a "last modified" date on the home page. To encourage user feedback and user interaction, I provided a mailing address and an e-mail address. Also important and located at the home page was a link to the government disclaimer.

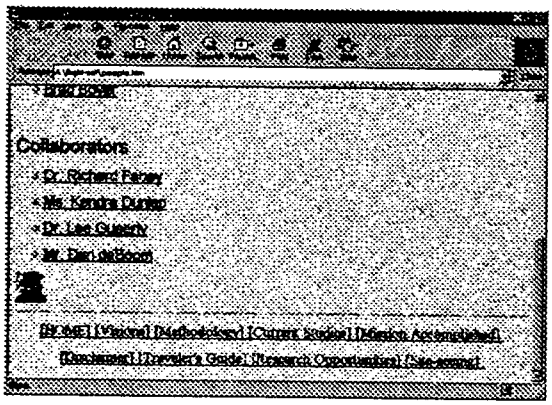


Figure 2. First draft, Personnel page in outline form.

I presented the Web collection to the CTA group at our first design review. Examples of user feedback included: a suggestion that one of the animations be frozen after a few rounds to prevent distraction, input on what links to related Web sites to include, and comments regarding the format of the pages, the titles on each page, and the proposed content. Plus, we assigned different sections for people to write and began taking photographs for the Web page.

I went back to the computer for revisions. The primary change was reformatting all pages (except the home page) into a tables-based interface. This allowed the modified menu bar on the bottom to appear the same on all pages, giving the Web site consistency. Also, I made head shots visible on the main Personnel page by changing the outline into a table (see Figure 3). In addition, I surfed the Internet for related Web pages that I could provide links to from this Web site. Gradually the Web pages expanded as people submitted information for me to include. With the help of a computer expert, I was able to have the animation in question freeze after a few rounds. In the second meeting, I presented the CTA team the revamped Web site. After some discussion, we decided that a CTA logo would make the site more attractive and coherent. One person remarked that the menu bar would be more noticeable if it were placed at the top instead of the bottom.

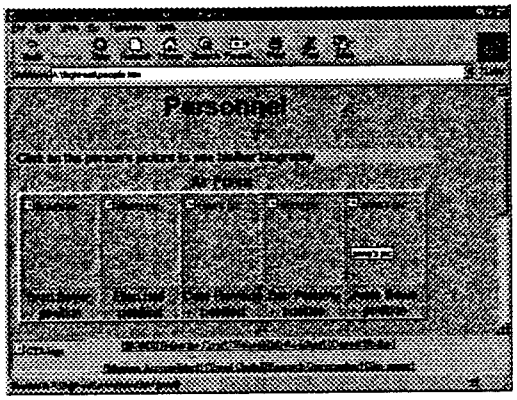


Figure 3. Second draft, Personnel page. Notice the revised (table) format, with spaces for pictures

Taking all the comments into consideration, I began the third draft. I decided the menu bar had to be altered. It was space consuming, unattractive, and not very noticeable. Also, the table-based structure of each page was not very efficient. After examining several Web pages containing frames, I decided frames would be the best

choice for our Web site. A frames-based interface, with its independently changeable viewing panes within the same browser window, has many advantages over tables. By setting up the frames, I was able to incorporate a Java applet based navigational* bar called CuteTabs. Placed at the top of the browser display, CuteTabs greatly facilitated site navigation, did not take up a lot of space, and was much more attractive than the old menu bar (see Figure 4). Also included in the static frame at the top was the CTA brain logo. This reminds viewers whose Web site they are browsing.


Figure 4. Third draft, Personnel page. Notice the CTA logo and navigation bar at the top.

Not only did the frames enable me to use a better navigation bar, it also eased the onus of page organization. I could program hyperlinks in one frame to update the contents of an adjacent frame. For the "Missions Accomplished" page (see Figure 5), viewers can scan the different project titles along the left side of the screen, click on one of interest to them, and have the abstract emerge in the main content area to the right.

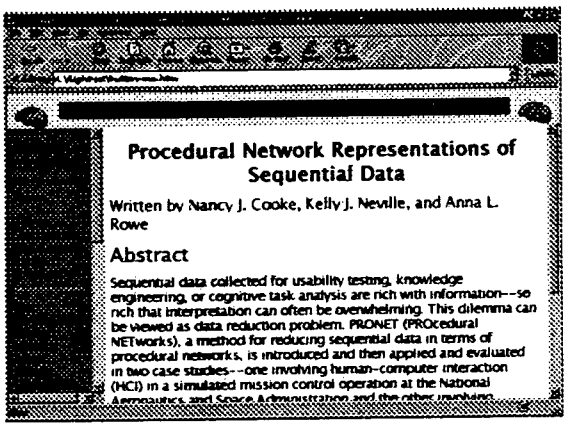


Figure 5. Third draft, Missions Accomplished page. Notice the frame on the left side showing project titles and the large frame on the right side, depicting an abstract.

With the majority of the work completed, I conducted some user testing on people who had never seen my Web collection before. I asked the subjects where they thought certain links would take them, based on the descriptions given. From this assessment, I learned my epithets were fairly effective, as all participants understood what type of Web page the various epithets described.

In addition, I conducted a "scavenger hunt," where I asked people to locate certain information on my Web site. I observed the subjects' navigation patterns to see if they were able to get to the target resource smoothly. This test alerted me to any potentially confusing areas. For instance, I noticed that users were having trouble getting back to the home page from Web pages that were at or near the bottom of the hierarchical structure. This prompted me to go back and add more navigation buttons to facilitate browsing. An interesting fact I noted was that users consistently referred back to the table on the home page instead of using the navigation bar.

## Discussion/Conclusion

Many lessons were learned as a result of the creation of the CTA Web site. Like Web pioneers, I found that establishing a Web site is a time-consuming, labor intensive, yet rewarding endeavor. Several weeks need to be set aside for planning, writing, testing, and revising.

I discovered manager support greatly facilitates group participation. Without a leader present to oversee progress, development can be slow and inefficient. In

addition, I learned first hand the importance of good organization, not only in regards to the Web structure, but also in keeping records and saving files.

It should also be noted that the successful Web sites are ones that are regularly being revised. Periodic updating and editing will aid in having old users return and entice new ones to visit. The benefits of a Web presence also comes with its responsibilities. Developers must make sure the Web pages they author serve specific purposes so they do not end up as another useless site.

From creating the CTA team Web site, I conclude that the product development cycle is a dynamic yet structured process. Although there are times that require creativity and ingenuity, basic, highly iterative steps exist that all developers follow. These are, namely: identifying goals, target user population, and constraints, prototyping of initial information structure and screen formats, evaluating the interface, redesigning, testing, implementing (Helander, 1991).

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Appendix

Good sites to visit

http://www.visdesigns.com/design/commandments.html
10 commandments for writing HTML, has good tips

http://www.ncsa.uiuc.edu/General/Internet/WWW/HTMLPrimer.html
good tutorial

http://www.nashville.net/~carl/htmlguide/index.html
How do they do that with HTML? Tips and tricks

http://www.mcp.com/general/workshop/
Tutorials for beginning, intermediate, and advanced levels

http://www.willcam.com/cmat/html/crossref.html
Compact index of HTML tags

http://www.webtechs.com/html-val-svc/
"HTML validation service"

http://home.netscape.com/assist/net_sites/index.html
"Creating NetSites" links to pages on creating netsites

http://www.cs.cmu.edu/~tilt/cgh/
"Composing Good HTML"- concepts to consider when writing Web pages

http://www.netscape.com/assist/net_sites/frames.html
An introduction to frames

tools to download

**SnagIt Twin Pack, Version 4.0**

SnagIt allows users to capture an entire screen, an individual window, entire contents of a scrolling window, or a user-defined portion of the screen. The captured image can be saved as a graphics file in .BMP, .PCX., .GIF, .JPG, or .TIF format, or it can be sent to the printer or clipboard. New features in this updated version include Text Capture, which lets you capture only text information on your screen, and Video Capture, which allows you to create a movie of events as they happen on your screen. The screen captures in my report were done with SnagIt.

Find it at: http://www6.zdnet.com/cgi-bin/texis/swlib/hotfiles/info.html?fcode=0009N2
Compressed Size: 741,871 bytes
Requirements: Windows 3.1x
Purchase Information: Shareware- free to try (for 45 days), $39.95 if you decide to keep it.

**Super NoteTab**

This handy 32-bit text editor allows users to edit multiple text files at once using a tabbed, multipage interface. Features include a fully configurable toolbar, page numbering, text statistics, a favorite file list, and a special Clipbook window with templates for HTML tags, acronyms, and smilies. Super NoteTab is web enabled, meaning you can open links and HTML documents in your browser, making it an indispensable program for Web authors.

Find it at: http://www6.zdnet.com/cgi-bin/texis/swlib/hotfiles/info.html?fcode=000CP6
Compressed Size: 732,581 bytes
Requirements: Windows 95
Purchase Information: Free

GIF Construction Set
This collection of tools enables you to create and edit multiple-block GIF files. Included in this graphics program is an Animation Wizard that assembles animated GIFs, perfect for use on Web pages. Other helpful features include AVI to GIF conversion, facilities to manage palettes, and the ability to create transparent GIF files.

Find it at: http://www6.zdnet.com/cgi-bin/texis/swlib/hotfiles/info.html?fcode=00033L
Compressed Size: 732,446 bytes
Requirements: Windows 3.1x
Purchase Information: Shareware- free to try, $20 if you decide to keep it.

Photo Line 32
Photo Line 32 gives you powerful image manipulation, with its masking, filters, layering, and special effects. I found it very effective for cropping pictures.

Find it at: http://www6.zdnet.com/cgi-bin/texis/swlib/hotfiles/info.html?fcode=000FZ9
Compressed Size: 1,687,128 bytes
Requirements: Windows 95
Purchase Information Shareware: Free to try, $69 if you decide to keep it.

LView Pro (32 bit)
This image editor, specially designed for Internet usage, has many features. Images can be rotated clockwise or counterclockwise. Files can be saved under different formats, like GIF, BMP, JPG, and TGA.

Find it at: http://www6.zdnet.com/cgi-bin/texis/swlib/hotfiles/info.html?fcode=00000H
Compressed Size: 1,304,290 bytes
Requirements: Windows 95
Purchase Information: Shareware- free to try, $40 if you decide to keep it.

# STUDY OF FACTORS INFLUENCING INJURY POTENTIAL ASSOCIATED WITH EMERGENCY EGRESS

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**Final Report for:**
**High School Apprentice Program**
**Armstrong Laboratory**

**Sponsored by:**
**Air Force Office of Scientific Research**
**Bolling Air Force Base, Washington, DC**

**And**

**Armstrong Laboratory**

**August 1997**

# STUDY OF FACTORS INFLUENCING INJURY POTENTIAL ASSOCIATED WITH EMERGENCY EGRESS

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## Abstract

The injury potential of crewmember populations possessing the greatest risk was established and assessed. Factors influencing injury potential were sex, age, and weight. Those populations which demonstrated the greatest injury potential were females, crewmembers above age 20 ( risk increases with age), and lower-weight crewmembers.

# STUDY OF FACTORS INFLUENCING INJURY POTENTIAL ASSOCIATED WITH EMERGENCY EGRESS

Kavitha K. Reddy

## Introduction

The Escape and Impact Protection Branch of Armstrong Laboratory, in cooperation and coordination with other research facilities, has pursued its goal to " assure safety and effectiveness of aircrew members by reducing fatalities and major injuries associated with emergency escape and impact." The pursuit of this goal is necessitated by the high injury potential associated with emergency egress, specifically by the use of the ejection seat, which exposes the escaping crewmember to potentially detrimental G forces at several points throughout the process. The most common injuries resulting from exposure to these forces are fractures of the spine and otherwise harmful head and neck injuries. In order to design protection devices, it is necessary to determine the population(s) having the greatest injury potential.

## Methodology

Several experimental methods have been employed in recent years to test the efficacy of protection devices and to establish injury criteria. The most common test devices generate G forces in the direction of one or more of the anatomical axes ( $\pm G_x$, $G_y$, $G_z$ forces). Test devices generally fall into one of three categories: 1) relatively simple towers used to drop test carriages onto decelerators, such as metal deforming devices or hydraulic cylinders, 2) horizontal tracks with various propulsion systems used to propel test carriages onto decelerators, and 3) high-pressure gas actuators used to accelerate a test carriage along either vertical or horizontal rails.(2) The latter, the Horizontal Impulse Accelerator, allows for the most impact control and reproducibility, and is presently part of the facilities found at WPAFB. Test subjects included anthropomorphic dummies (particularly the ADAM, or advanced dynamic anthropomorphic dummy) , rhesus monkeys, cadavers, and humans. The investigator must be mindful

when comparing data from distinct test facilities that all possible variances, including body support and restraint system configuration, restraint pretension, subject bracing, similarity of the acceleration waveform, and pre-test conditions, are taken into account. Test data has been analyzed and manipulated using a variety of models.

## Results

The results are summarized in the following graphs.

Figure 1 relates the rate of spinal injury to DRI, or the dynamic response index. The DRI is an index of thorax displacement.

Figure 2 demonstrates how DRI is affected by the weight of the pilot. It can be seen that DRI and crewmember weight are inversely related.

Figure 3 provides data supporting the conclusion that females have lower neck strengths than males.

Figure 4 summarizes reviews of literature as reported in Burhman (3).

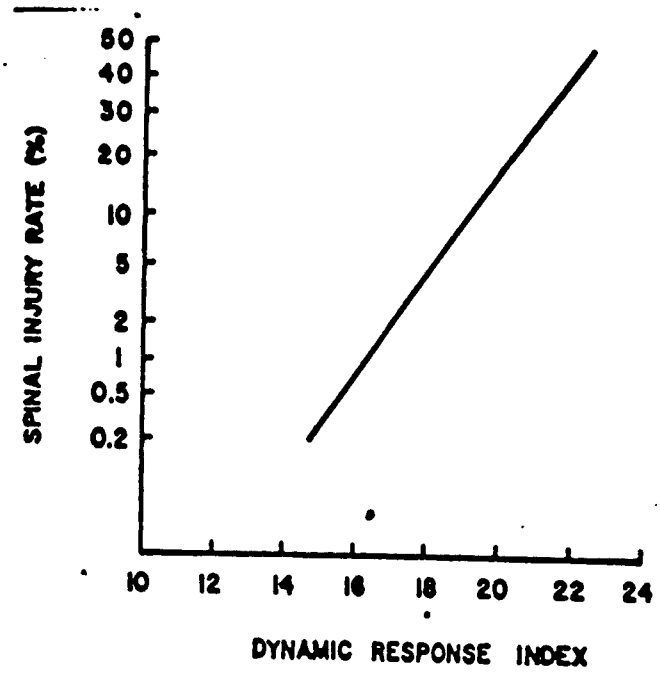
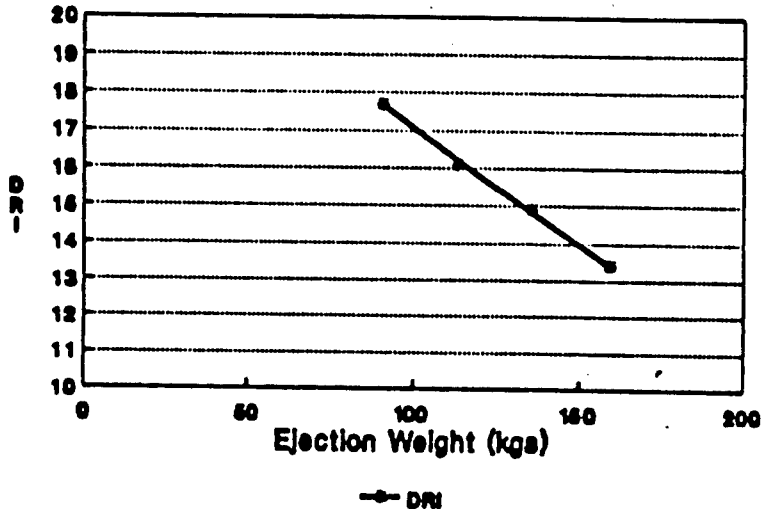
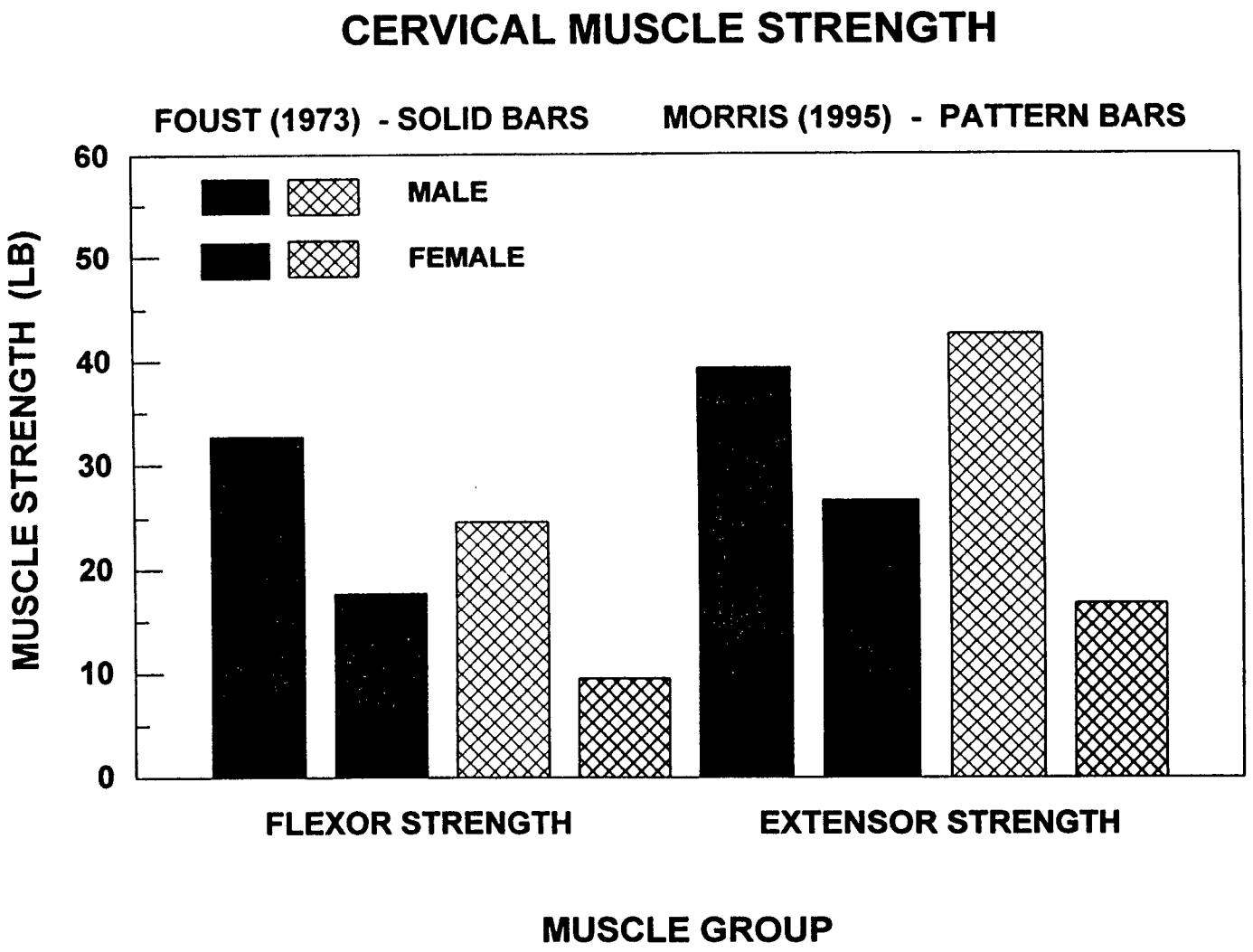



FIGURE 2. DISTRIBUTION OF SPINAL INJURY RATE



# HMS: MALES/FEMALES



# HMS: MALES/FEMALES

MALE AND FEMALE RESPONSE:

- LITERATURE REVIEW / FEMALE INJURY



  - 25% GREATER RISK OF INJURY IN AUTO FATALITIES (Evans)
  - 18% DECREASE IN RIB STRENGTH (Foret-Bruno)
  - 18-30% DECREASE IN LUMBAR VERTEBRAL BREAKING STRENGTH (Hansson)
  - 17% DECREASE IN VERTEBRAL BREAKING STRENGTH IN COMPRESSION, TENSION, AND TORSION (Yamada, Sonoda)

## Conclusion

Populations having the greatest injury potential associated with emergency egress were established. Injury potential is at a minimum at the age of 20 years, and increases with age. For each year after 20, the risk of injury increases by 2.3% for males, and 2.0% for females. It is also concluded that females have a 25% greater injury potential than males from the ages of 15 to 45 years. One factor of this increased injury potential may be low body weight. It has been shown that an increased risk of spinal injury exists for crewmembers whose weights fall below the 5th percentile (a 140 lb. male pilot). The significance of this factor becomes apparent when one considers the fact that nearly one half of female Air Force pilots fall into this category. Injury potential appears to increase with decreasing weight. This is because the acceleration of the ejection seat during the catapult (rocket-burning) phase increases when the seat is occupied by crewmembers of low weight. Further investigation is recommended into the effects of sex, age, and weight on injury potential, as well as new factors, such as Head-Mounted devices (HMDs). Advances in aerospace technology have magnified the importance of impact protection efforts by consistently increasing the complexity and danger of the environments crewmembers face. For this reason, Escape and Impact Protection research and development will continue to play and vital role in Air Force operations.

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Warminster, PA 18974-0591

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# A Study Of The Methodology Used In An Experiment Testing The Effect of Localizing Auditory Signals On The Subject's Ability To Track Information

Anitha Reddy

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
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Wright-Patterson Air Force Base, OH

and

Armstrong Laboratory

August 1997

# A Study Of The Methodology Used In An Experiment Testing The Effect Of Localizing Auditory Signals On The Subject's Ability To Track Information

Anitha Reddy
The Miami Valley School

Abstract

A study addressing the influence of localization on a subject's ability to detect, identify, and monitor several auditory signals simultaneously was begun. Four men and four women were used to make the initial recordings, short phrases including standard military call signs. This paper documents the methodology used to make these recordings, which would eventually be localized and used in the experiment. It also details some of the initial processing the recordings underwent to control for irrelevant variables such as amplitude and length before the recordings were localized.

# A Study Of The Methodology Used In An Experiment Testing the Effect of Localizing Auditory Signals On The Subject's Ability To Track Information

Anitha Reddy

## Introduction

As a pilot's capacity for information intake through the visual channel reaches its maximum, researchers have become increasingly interested in the advantages of using the auditory channel as a pathway for auxiliary information. Using localized auditory signals can offer more than just simple content information by their very nature. With localization, signals can indicate location as well. Whether the location of the auditory information corresponds to a pilot's target or is used as a warning system, the advantages associated with localization clearly require further research.

The purpose of this study is to test participants' ability to monitor up to eight spatialized auditory signals concurrently. The objective is to discover whether or not the spatial orientation of auditory information can increase the pilot's capacity to successfully track large amounts of information at the same time. In this study participants' heads would be centered in a geodesic sphere equipped with speakers. To prepare the auditory samples eight subjects, four men and four women recorded phrases with a high face validity for operational environments. These phrases included standard terminology used when communicating with pilots. The auditory samples will be localized in an anechoic chamber equipped with a geodesic sphere. At the time of this paper's publication, sample collection was completed and processing had begun. The purpose of this paper is to discuss the methodology involved in the data collection for this study and the reasoning behind the initial stages of the data processing.

## Methodology

The initial recordings were made in a quiet room, with its sound reflective surfaces minimized. It was lined on five walls with acoustic foam and the floor was lined with carpet. A .5 inch diameter Bruel & Kjaer microphone was used. It was covered with a wind screen and was Type 4165. This was connected to Bruel & Kjaer Type 5935 power supply and preamplifier. This in turn was connected to a Tucker Davis DD1 combined digital to analog and analog to digital converter. The converter and the computer were

connected by a fiber-optic interface. The recordings were stored in digital samples on the computer's hard drive in speech files.

Three men and two women were selected from the laboratory's subject pool to make the initial recordings. For a total of eight talkers, two female summer interns and a programmer employed by the base made recordings as well. Each participant recorded a set of sentences that included standard call signs. An example of one of the sentences is "Ready, Hopper, go to blue 1 now." Each participant recorded this basic sentence 256 times with the following variations. A specific call sign would be used in 32 sentences, but the color and number in the sentence would change. Each color would be used in a sentence with a call sign and a one number between 1 and 8. Eight call signs (Hopper, Baron, Charlie, Ringo, Arrow, Laker, Tiger, Eagle) and four colors (red, blue, white, green) were used. In addition to these sentences, the participants were also required to record forty true or false sentences to be used in a Sentence Verification Test, whose intelligibility had already been confirmed by a previous study.

To eliminated as much unnecessary variation as possible in the talkers' voices, they will all characterized and reconfigured to a new amplitude, the average amplitude of all of the talkers' voices. To accomplish this the dead space on each recording had to be eliminated, as it would artificially lower the average amplitude of that particular recording.

The dead space at the beginning of each recording was eliminated digitally using an automatic algorithm that detected the start of each sentence and chopped of the recording of silence preceding it. The dead space at the end of each recording was eliminated using a computer program which allowed the user to chop off dead space in 8 digital samples. The program also allowed the user to restore a sentence if too much had been eliminated from the recording. Thus, if after some multiple of 8 digital samples the user found that she had eliminated part of the sentence as well, she could "undo" the cutting in 2 digital samples.

The machines used to achieve playback of the recordings for cutting purposes were the Tucker Davis DD1 converter and a Crown D-75 amplifier.

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# A STUDY OF THE SHIFTS IN SCENE PERCEPTION MEMORY

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
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and

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August 1997

# A STUDY OF THE SHIFTS IN SCENE PERCEPTION MEMORY

Esther I. Resendiz
W.H. Taft High School

## Abstract

In order to explain the phenomenon of vertical shifts in scene perception memory (Resendiz), an experiment was conducted to study the perspective shifts and compression of objects. Two reasons were hypothesized to cause this vertical shift: there could be a shift in the memory of objects from space close by into more distant space or there could be an elevation of the head to a default position in memory. Six scenes with a central point of interest were photographed, each from 0°, 30°, and 60° viewpoints. Thirty subjects viewed a photograph of each scene at various angles for fifteen seconds each. The subjects were then immediately allowed two minutes and fifteen seconds to redraw the pictures exactly as they remembered them. The following measurements were then made on the drawings: the compression of the objects, the vertical shift in the points of interest, and the shift in certain perspective angles. All of the drawings showed similar object compression and, additionally, the drawings of 0° and 30° scenes demonstrated a downward vertical shift. Generally, subjects tended to adjust their perspective to a default head position; thus, the objects drawn reflected the normal viewing angle at which one would see the objects in the picture.

# A STUDY OF THE SHIFTS IN SCENE PERCEPTION MEMORY

Esther I. Resendiz

## Introduction

Although many people believe their memory to be accurate, there appear to be several universal biases concerning the perception of scenes that are remembered. Such shifts in scene perception memory could be responsible for a pilot's faulty perception memory in relation to a runway or features in the terrain. Past research (Resendiz) has revealed a phenomenon of vertical shifts and, in an effort to understand the implications of this, it is important to understand why this shift occurs. The difference between peripersonal and extrapersonal space, regions in space studied by Previc (Previc), may help to explain the reasons for some of the perception shifts. Peripersonal space is the area directly around a person, the area within their "grasping" distance. Generally, this space concerns us more and tends to be at an angle of depression from our eyes. Extrapersonal space includes area further out, like objects viewed along the horizon (See Figure 1). This space is associated more with the upper visual field, since this is where objects in the distance are more likely to be located. One reason that shifts in perception may occur is because we tend to remember objects as being further away from us, and therefore, the upper visual field expands to complete our mental picture. Another reason that would explain such shifts is the fact that people may remember scenes as being viewed from their default head position. This means that we remember objects from angles at which we are accustomed to seeing them. As we elevate or depress our head position in memory, there is a vertical shift accordingly. An experiment was designed in order to reveal additional distortions in scene memory would support one of our hypotheses.

## Methodology

Six scenes were photographed, each at 0°, 30°, and 60°. The 30° was considered to be the "natural", or the normal position at which one would be accustomed to viewing the objects. In addition, each photograph contained a central point of interest. The eighteen pictures were divided into three sets, each

set containing varying angles of the same six scenes. Thirty subjects were then divided into six groups of five and each set of photographs were shown to two groups, one in regular and one in reverse order. The subjects had fifteen seconds in which to view slides of the photographs immediately after which they had two minutes fifteen seconds to recall the scene from memory and redraw it in a 4" by 6" box on a page in a booklet. (See Appendix).

Since the points of interest in the originals were centered, the points of interest in the subjects' drawings were then measured against the originals by measuring the percent they varied from the mid-line. The compression of the objects was measured by taking the width of an object common to the original and the majority of the drawings, by converting it to a measurement of the percent of the drawing that it occupied, and by comparing the percent against the original picture. The perspective of the viewer was judged by the way they drew a circular feature or chosen angle in each picture. The perspective for the circular features were measured with a vertical to horizontal aspect ratio by taking the height of the feature, dividing it by the width, and then converting it to a percent which made it comparable to the original. This way, 0% was considered the head-on view and 100% was considered the God's-eye view. To maintain consistency, the perspective angles at the top of objects were measured and the head-on view, (a 180° flat line), was considered to be 0% and the God's-eye view, (a 90° angle), was considered to be 100%. The percents of the angles in between were then figured accordingly. The perspective measurements from the drawings, in the form of percents, were then compared to the perspective measurements in the originals.

Results

The perspective change of the circular tops (i.e., aspect ratio) for the 0° pictures was a 19% elevation, for the 30° pictures there was a 4% depression, and for the 60° pictures there was a 17% depression (See Figure 2). The perspective change of the perspective angles for the 0° pictures was a 31% elevation, for the 30° pictures there was a 6% elevation, and for the 60° pictures there was a 4%

depression (See Figure 3). Overall, the perspective change of the 0° pictures was a 27% elevation, for the 30° pictures there was a 3% elevation, and for the 60° pictures there was an 8% depression (See Figure 4). The points of interest shifted downward 8% in the 0° pictures, they shifted downward 15% in the 30° pictures, and there were no (0%) vertical shifts in the 60° pictures (See Figure 5). The compression of the objects shot at 0° was 3%, the 30° pictures were compressed by 7%, and the 60° pictures were also compressed by 7% (See Figure 6).

Discussion

The shifts in scene perception memory seem to be biased towards the lower field, accompanied by the compression of objects, and the perception seems to default to an angle of depression at which one might view an object on a table. The compression of the objects did not significantly differ between the 0°, 30°, and 60°. Though it is unclear why the 30° views showed the greatest downward vertical shift, the 60° views showed a strikingly different vertical shift trend than the pictures as a whole as they did not vertically drop but stayed the same. The possibility that the vertical shifts occur because of the shifts of objects in memory from peripersonal into extrapersonal space seems less likely because the compression of the scene occurred for all viewing angles and no vertical shift occurred in the 60° view.

Although the vertical shifts didn't directly coincide with the perspective shifts, there is enough correlation to make a reasonable conclusion that the assumed head rise in memory and the vertical drop are related. The fact that the head-on views were remembered as being more elevated, the 30° views were remembered as being elevated only a minuscule amount, and the 60° views were remembered as being more depressed clearly implies that subjects remembered objects as being at a "comfortable", normal depression from their eyes. Due to the shifts in perception, a default head position is extremely probable.

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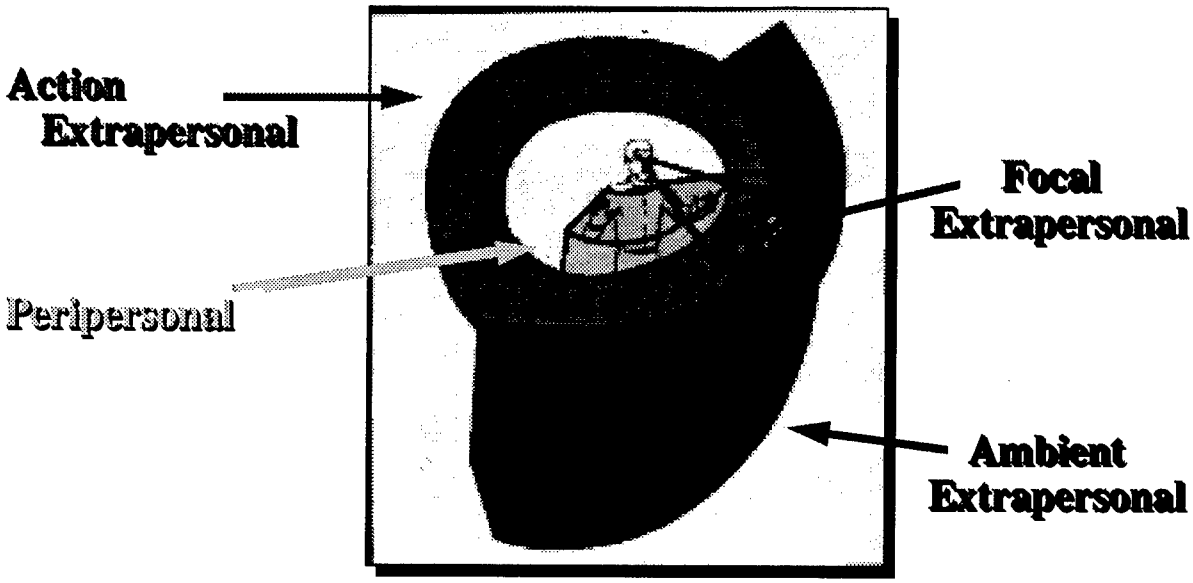
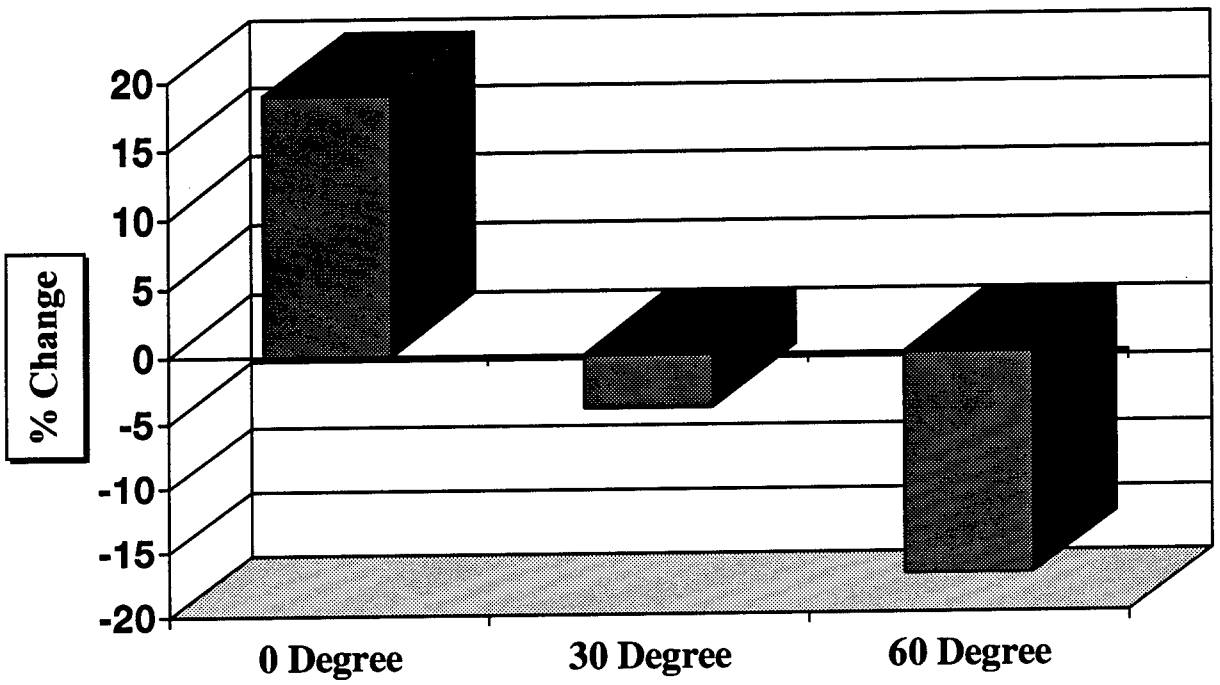
Figure 1

# 3-D Behavioral Systems



## Figure 2

# V-H Aspect Ratios



## Figure 3

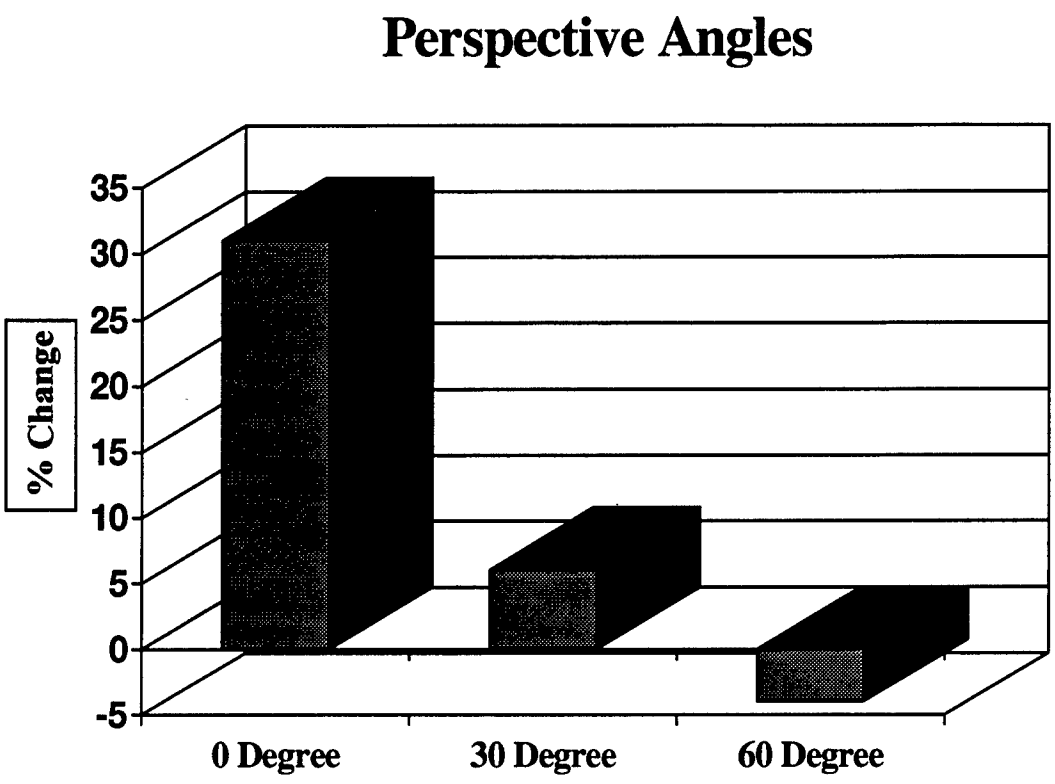

Perspective Angles
% Change
35
30
25
20
15
10
5
0
-5
0 Degree
30 Degree
60 Degree

**Figure 4**

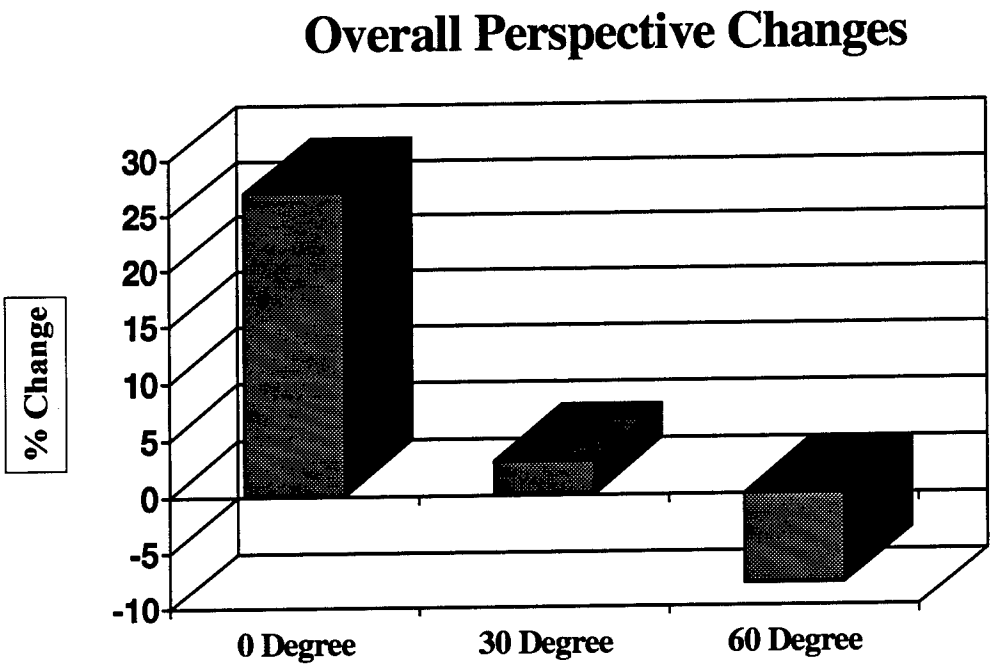

Overall Perspective Changes
% Change
30
25
20
15
10
5
0
-5
-10
0 Degree
30 Degree
60 Degree

**Figure 5**

# Vertical Shift

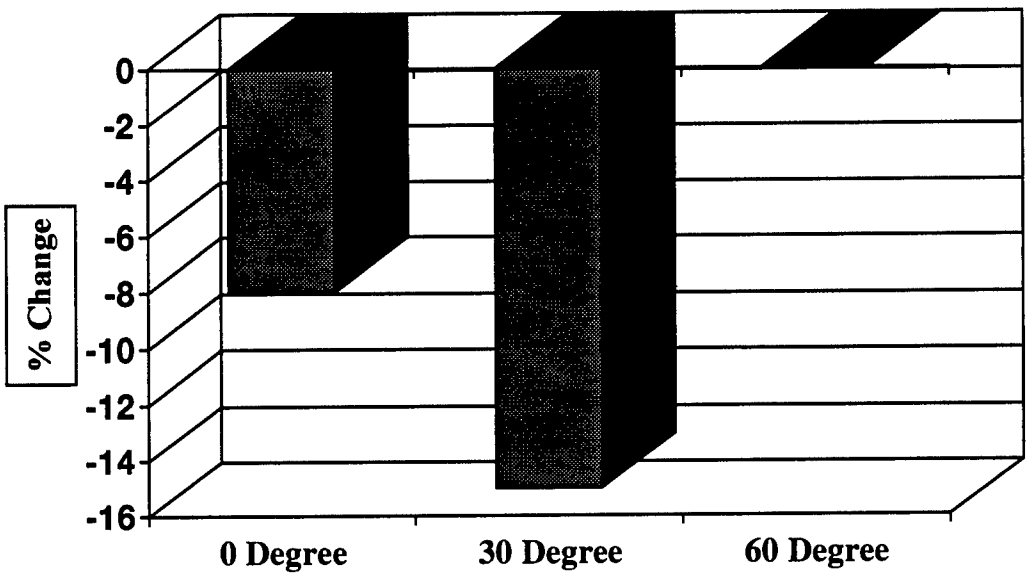
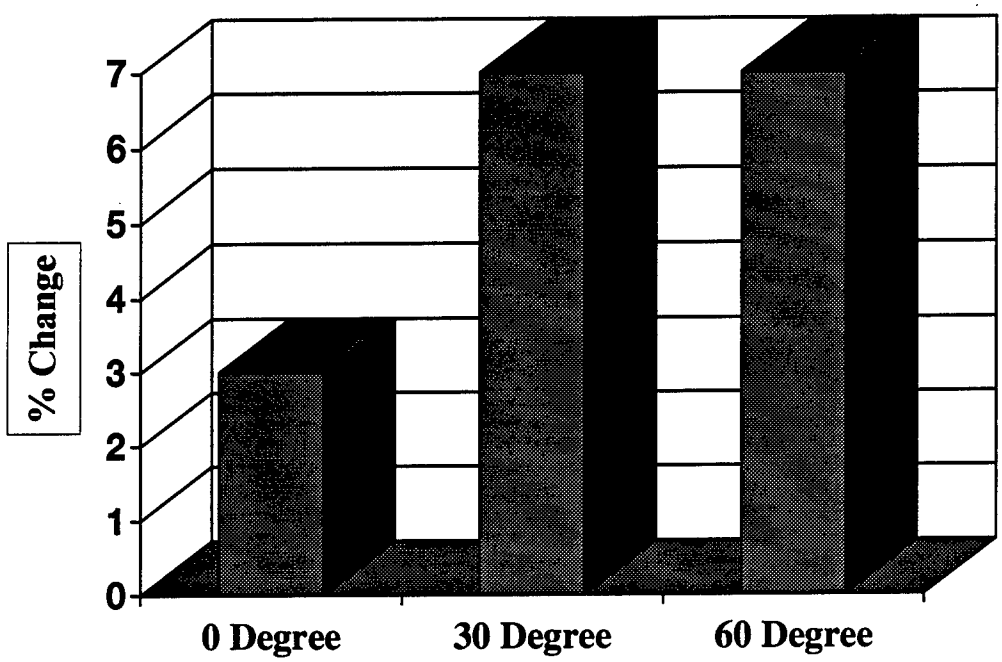


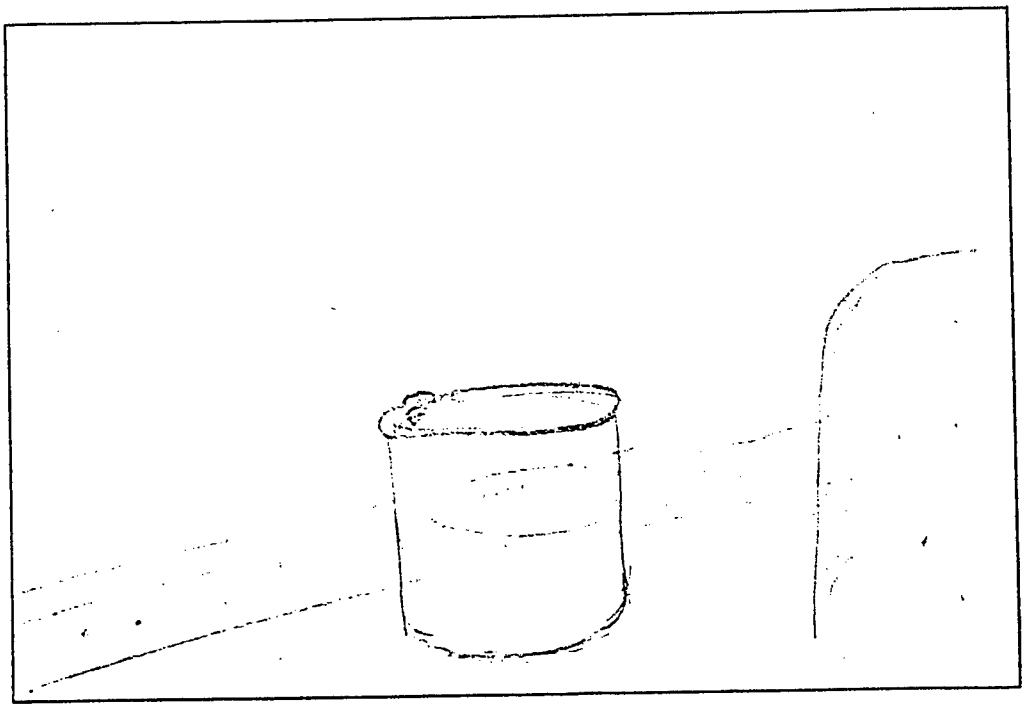
**Figure 6**

# Compression



Trash Can

0° View



**Associate did not participate in the program.**

# A STUDY OF THE ANALYSIS OF URINARY BENZODIAZEPINES USING ENZYME HYDROLYSIS

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Final Report for:
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and

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# A STUDY OF THE ANALYSIS OF URINARY BENZODIAZEPINES USING ENZYME HYDROLYSIS

Rachel A. Sharp
William Howard Taft High School

## Abstract

**An alternative procedure to acid hydrolysis for extracting benzophenones was investigated in order to achieve greater ease and accuracy during identification. A solid phase extraction and GC-MS confirmation method was used. Samples were hydrolyzed with β-glucuronidase at 37°C, extracted with Bond Elut Certify ® columns, and dried. Sample preparation time averaged 1 to 1 1/2 hours. Using this procedure, we are able to more accurately report the benzodiazepine most likely ingested by the patient.**

# A STUDY OF THE ANALYSIS OF URINARY BENZODIAZEPINES USING ENZYME HYDROLYSIS

Rachel A. Sharp

## Introduction

Ever since the first benzodiazepines were synthesized in 1955 during a search for a patentable tranquilizing drug, researchers have been continually striving to produce new and more versatile ones {1}. More than 3000 benzodiazepines have been synthesized and at least 25 are commonly used in countries throughout the world. Among the most widely used in the United States are Alprazolam (Xanax), Diazepam (Valium), Clorazepate (Tranxene), and Lorazepam (Ativan) {2}.

There are four primary pharmacological uses of benzodiazepines including use as an anxiolytic agent, as a muscle relaxant, anticonvulsant, and as a sedative-hypnotic. Some unfortunate side effects of many benzodiazepines are drowsiness, loss of muscular coordination, and muscular weakness {1}. Use of these drugs on a regular basis can possibly lead to an addiction and withdrawal symptoms that may last for weeks {3}. Benzodiazepines are the most widely prescribed psychiatric drugs. They are most often used to treat maladies as sleep disorders, anxiety, alcohol withdrawal, and seizure disorders {3}. In 1987, it was estimated that about 10% of Americans took a benzodiazepine {2}.

As the estimated use of a particular drug increases, it is inevitable that the instance of its abuse will also accordingly increase. This makes it necessary for drug testing labs to have an effective procedure to detect a particular drug. Because of the astronomic increase in the variety of benzodiazepines, a

simple indicator test is not sufficient for their accurate identification. Drug testing labs need an efficient and effective mode of testing for the presence of benzodiazepine metabolites so they can accurately report the exact benzodiazepine being used.

Labs can use a Fluorescence Polarization Immunoassay screening to initially test urine for the presence of benzodiazepines and/or their metabolites. Those urines which test positive are evaluated further to confirm the existence of benzodiazepines and identify the specific benzodiazepine ingested.

The human body usually conjugates a benzodiazepine metabolite before excretion. An acid treatment (hydrolyzing the conjugate) is quite useful for identifying the corresponding benzophenone. A gas chromatography/mass spectrometry analysis testing an acid hydrolyzed urine specimen will confirm the presence of a benzodiazepine metabolite and sometimes identify the parent drug.

However, several benzodiazepines may be metabolized to the same metabolite and because some

metabolites produce the same benzophenone; therefore, the acquisition of more specific data requires further analysis. Definitive confirmation of the benzodiazepine can be accomplished through enzymatic hydrolysis and identification of the unconjugated benzodiazepine in the urine.

## Discussion of Problem

In order to properly identify specific benzodiazepines accurately, we needed a better procedure for extracting them from patient samples. Our acid hydrolysis procedure was too destructive toward the benzophenones. Acid hydrolysis breaks down the metabolites to such an extent that identification of the parent drug becomes a virtual impossibility. We investigated a procedure utilizing the enzyme β-glucuronidase that, under the right conditions, deglucuronidates the conjugated metabolite. This frees it so we can then extract the benzodiazepine drug and identify them using gas chromatography - mass spectrometry (GC-MS).

## Materials and Methods

*Reagents.* β-glucuronidase from E. coli was obtained from Sigma Chemical Company. Bond Elut Certify® solid phase extraction columns were obtained from Varian. Bovine Serum Albumin was obtained from Sigma Chemical Company. Oxazepam glucuronide (100 mg/ml) was obtained

A STUDY OF THE ANALYSIS OF URINARY BENZODIAZEPINES USING ENZYME HYDROLYSIS

Rachel A. Sharp

from Alltech.

*Equipment.* A Finnegan GCQ was equipped with a A200S autosampler and computer instrumentation control with data analysis software package, including Selected Reaction Monitoring (SRM), Selected Ion Monitoring (SIM), and ECD-MS with both EI and CI operational modes. The instrument is autotuned weekly and injected daily with a functional standard composed of Nicotine, Caffeine, Codeine, and Quinidine. The injection port temperature is 275°C, and the interface temperature was held at 275°C. Urinary benzodiazepines are screened using the Fluorescence Polarization Immunoassay (Abbott) using an Abbott AxSym and are assayed using thin layer chromatography (ToxiLab, Inc.)

*Chromatographic Conditions.* Samples were placed in injection vials and assayed on the GC-MS using a 12.5m x 0.2mm i.d. methyl silicone fused silica capillary column. The sampler was rinsed 6 times with chloroform following each injection. We used a split ratio of 30:1; temperature started at 140°C and ramped at 30°C/sec to 260°C. The carrier gas was helium and the flow rate was 40 cm/sec.

*Standards.* A stock solution (100 μg/ml) of an Internal Standard of Benzphetamine was made in deionized water. A functional standard with Nicotine, Caffeine, Codeine, and Quinidine was used. A positive control using oxazepam glucuronide from Alltech was made by spiking negative urine

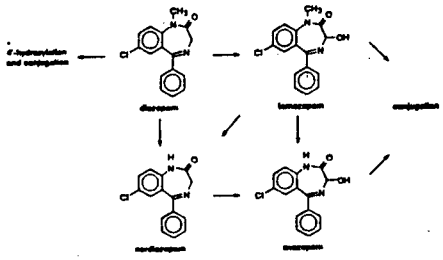
control.

*Methodology.* A positive control is made by adding 50 µl of 100 µg/ml oxazepam glucuronide to 5 ml negative urine control (final concentration 200 ng/ml oxazepam glucuronide). 5 ml of negative urine is used for the negative control. 100 µl of internal standard is added to the controls and to the patient samples. Phosphate buffer with 1% Bovine Serum Albumin and 1 unopened vial of β-glucuronidase was allowed to equilibrate to room temperature. To the 5 ml urine with internal standard, the entire contents of a buffered vial of enzyme (1000 Fishman units) and 1 ml of 0.1 M phosphate buffer with 1% Bovine Serum Albumin (pH 6.8) were added. The samples were then incubated at 37°C for 45 minutes. Note that the use of a cold buffer and/or preheated water bath could denature the enzyme. The urine was then extracted using Bond Elut Certify® Columns and the Vac Elut System. The columns were prepped by drawing through 3 ml methanol, 3 ml deionized water, and 1 ml 0.1 M phosphate buffer (pH 6) at full vacuum without drying out the sorbent bed. The samples were given 2 minutes at approximately 5 psi to flow through the columns. The columns were then rinsed with 2 ml deionized water, 2 ml 20% acetonitrile in 0.1 M phosphate buffer (pH 6). The column was then dried for 5 minutes at greater than 10 psi. The column was then rinsed by drawing 2 ml hexane through under full vacuum. The drugs were then eluted with 3 ml ethyl acetate by centrifuging at

# A STUDY OF THE ANALYSIS OF URINARY BENZODIAZEPINES USING ENZYME HYDROLYSIS

**Rachel A. Sharp**

**Figure 1 - Chemical structure of Diazepam and its metabolites.**



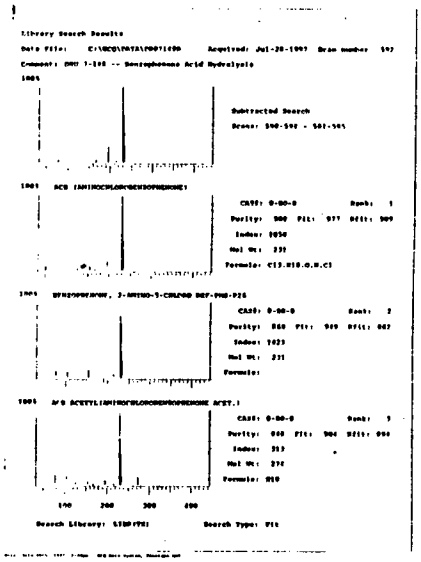
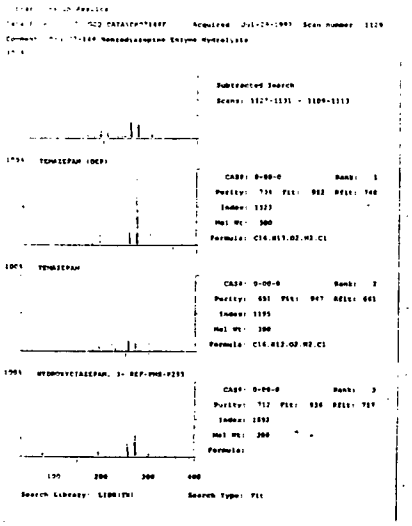
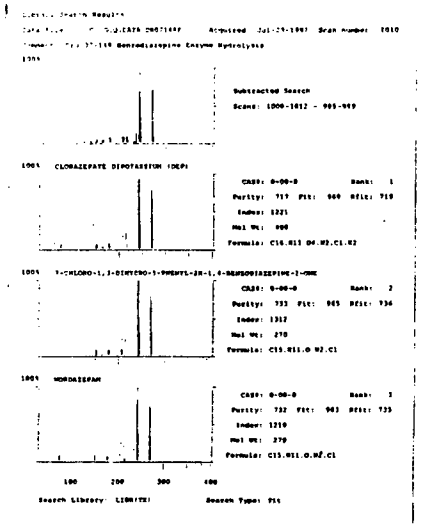
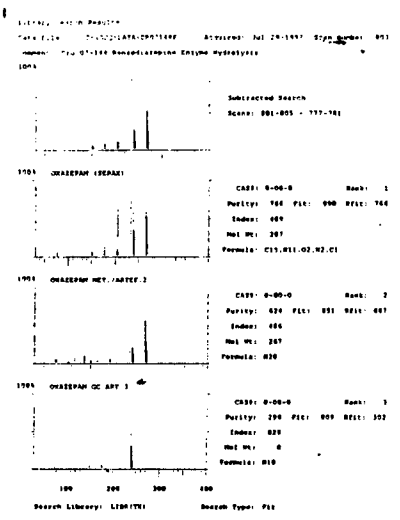


**Figure 2 - GCQ printout of acid hydrolysis results.**

**Figure 3 - GCQ printout of enzyme hydrolysis results.**







300 rpm for 3 minutes. Samples were then evaporated till dry in a warm water bath (approximately 40C°) with air flow. The samples were then reconstituted with 100 µl of chloroform and 1 µl was injected into a GCQ autosampler.

## Results

With this enzyme hydrolysis procedure, we found that we could frequently identify the parent drug that created the metabolites we extracted. For example, in patient #1, we noted three significant benzodiazepine peaks on the mass spectrometer at 803, 1010, and 1129. At 803 the substance is identified as oxazepam. At 1010, the substance's peaks were most closely matched to that of nordiazepam. At 1129, the computer chose temazepam as the closest match to that substance. With these three peaks, we found the parent drug to be temazepam and the primary metabolites nordiazepam and oxazepam {1}. The most specific our former acid hydrolysis procedure could get was diazepam metabolites, which really leaves a lot of questions as to what the identity of the parent drug is. If we compare Figures 2 and 3, we find that there is a larger amount of ease in reading and understanding the mass spectra. Despite the success of this procedure, we still ran into some problems, particularly with patient samples #2 and #3. In the first patient sample there were no matches for the mass spectra we found, but we think it is α-hydroxyalprazolam and are

trying to find a match for it. In the second sample, there wasn't enough of any metabolite to detect and identify. Its FPIA was barely over the cutoff. In cases like these, there isn't enough of a concentration for us to extract effectively. We can get a positive result on the FPIA (Fluorescence Polarization Immunoassay), and still not be able to identify the parent drug because of low quantities. These shortages of measurable drug are fairly common because only about 3% of the parent drug is excreted unchanged into the urine {1}.

## Conclusion

We conclude that an enzyme hydrolysis procedure using β-glucuronidase is a superior method of extracting benzophenones when compared to an acid hydrolysis procedure. Its success is clearly shown when we compare the mass spectra of the acid hydrolysis to those of the enzyme hydrolysis. The benzophenones can be clearly identified, in most cases without the use of algorithms and other exhaustive means of research.

A STUDY OF THE ANALYSIS OF URINARY BENZODIAZEPINES USING ENZYME HYDROLYSIS

Rachel A. Sharp

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James Sovel's report was not available at the time of publication.

# ABDR: REMOTE ENGINEERING REQUESTS

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**Final Report for:**
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# ABDR: Remote Engineering Requests

**Curtis J. Sparks**
**Nazarene Christian High School**

## Abstract

Remote engineering requests were studied for the purposes of solidifying a new system for these procedures in Aircraft Battle Damage and Repair. Computer communications were envisioned for this system, and that is where the demonstration system went this summer for us as high school apprentices. The demonstration system for remote engineering request utilized computer conferencing software aided by various other software and hardware applications such as annotation software, e-mail, and Netscape Navigator, amongst other programs. An instruction process was given to follow between the assessor of the damaged aircraft and the engineer who is trying to be contacted remotely by the assessor. Our team project made a demonstration of remote communication between assessors of aircraft battle damage and engineers capable of fixing abnormal repair.

# ABDR: Remote Engineering Requests

Curtis J. Sparks

## Introduction:

Aircraft Battle Damage and Repair (ABDR) has long been a vital part of the U.S. Air Force and it's top ranking position in the battle for air supremacy. The main objective of ABDR is to "enhance the wartime repair capability of aircraft maintenance activities...by assessing and/or repairing damaged aircraft in sufficient time to contribute to immediate wartime requirements."[1] For many years ABDR was treated as something different than what you see today. World War One recorded the first documentation of ABDR when parts of French farm machinery were used to fix damaged aircraft. As time passed by and World War Two came about ABDR began improvement. Through trial and error technicians found parts of aircraft that would temporarily fix the needed predicament. The system of ABDR in World War Two worked for the day in which it was needed but, with technology increasing in aircraft design and the complexity of parts, a more orderly system would be necessary. Vietnam was an eye opener for a whole new world of Aircraft Battle Damage and Repair. During this time many realized that supply of parts, speed of assembly, and communication were a vital in the ABDR process. US fixed wing aircraft had over 11,800 cases of aircraft battle damage from 1965 to 1975[2]. Since this era assessors, technicians, and engineers have tried improvement in various areas of ABDR methodology.

Through many long hours assessor/technicians have had to labour in fixing damaged aircraft to return them to battle capable condition. The repairs assessors/technicians perform are normally under harsh conditions and, even perhaps, in war-time environments. Through necessity aircraft should be kept highly populated at all times for a greater air-fighting force. This is the reason why speed is essential for the engineer and assessor/technician teams to get the aircraft in "back in shape".

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[1] Aircraft Battle Damage Repair for the 90's and Beyond; AFR 66-8,Equipment Maintenance: Aircraft Battle Damage Repair, 5 February 1992, 1.

[2] ABDAR Engineering Handbook ; August 1996.

Up until the present day assessors and technicians have recorded, assessed, and filed aircraft damage along with filling out documents such as AFTO 97, UXO ( Unexploded Ordinance) forms, and have sent data of the damage back to SERVIAC; all through a paper indexing system. These ways are fine and effective, although, the time factor plays an important role in today's ABDR. With the invention of micro-circuitry; the design of hand-held calculators, personal computers, and various other electronics have come into our everyday lives. The USAF has started to make use of this technology and has planned to use it in ABDR. Portable Maintenance Aids (PMA's) could be used to assist the assessor/technician in assessment and repair of damaged aircraft.

Prototype systems, for quickly assessing damage the aircraft has attained, utilize computers containing a fairly complex technical manual of a specific aircraft. These systems include IPDF(Index Portable Document Format) which is a system that electronically indexes the data and has "hot links" built into it to connect various topics associated with that specific command. The IETM (Integrated Electronic Technical Manual) is an interactive system of questions which would ultimately lead you to the correct assessment of the battle damage. The hypothesis of such systems is user friendly interface making for faster service in today's ABDR logic.

The engineer has long been part of the total package working ABDR along with assessor/technician teams. In the ABDR procedure(s), the engineer's job is to provide instruction for abnormal repairs of the damaged aircraft to assessor/technician, along with anything else essential to the repair of the aircraft, when the on-base engineer cannot perform the necessary task to get the aircraft in battle capable condition. This is a special job and requires the expertise of a skilled engineer to perform many tests to insure safety and stability of the aircraft. The engineer's role in ABDR is of great importance now and in the future of the program. Our job this summer will be to prove or disprove the idea for a communication advancement between the assessor(s) and the engineer(s) regarding remote engineering request for current ABDR.

**Communication - The intranet:**

Communication between the assessor/technician and engineer will best be utilized by an intranet containing a huge data base for all AFTO97 forms, aircraft data, and similar information. The use of the intranet will allow for quickly reaching either assessor or engineering parties. Engineers or assessors who are not skilled in the repair of a specific aircraft in need of fixing during war-time conditions can gain the assistance of another engineer, via intranet, from the nearest friendly base. The intranet provides for greater communication when the lack of ability of the on-hand engineer does not suffice for the needed repair.

The intranet will permit only a specific group of people to use a data base and the ABDAR web page that allows the assessment of aircraft electronically. The engineer could be reached by the same system of communication (the intranet) as the assessor/technician team doing the assessment. By the use of the intranet "mail systems", assessor/technician teams can contact an engineer over the web to do their calculations on a specified damage site on an aircraft. A strong system of communication to contact an engineer would be to send a request out to all engineers of a specific aircraft to see if anyone could respond to the call at the depot upon that time, or, if you wanted a specific engineer to work with, send out communication for that particular engineer.

**Research- software packages for aircraft repair assessment:**

Software development today is geared toward functions for certain operations performed by the user. The software that could be developed for the engineer would have to be designed to precisely what they needed. The development of new software for use by the engineer would cause for major expenses but could pay off in the long run due to the vitality of the product for ABDR.

Many people have enjoyed the use of E-mail, internet, and networks for either business or recreation. These systems quickly allow sending or accessing information on various topics. Many types of communication are allowed through the internet ranging from real-time video conferencing with speech capabilities to annotations given on

photographs by an individual then returned to the sender for evaluation. Possibilities like these for use in the USAF logistics division show that light has dawned on a new generation of ABDR.

Since the advent of the information super-highway, software developers and web surfers alike have seen various people voice their opinion through media and text. In certain cases, some sites on the internet allow only for specific programs to view their web page; most of the time not available on your personal computer at that time. Software companies have developed special tools called plug-ins which allow a internet user the ability to view or construct video, hear audio, and do numerous other applications on the internet. Plug-ins may prove to be useful to the future of ABDR due to its new plans for intranet based access to technical orders. Through the use of plug-ins the intranet can be used to view various pictures, video, or sound; whatever the engineer or assessors see fit to explain their problem or solution they hold.

Assessors find that quickly contacting an engineer is important in repair of abnormal aircraft damage. What if the engineer on base is not skilled in the repair of the aircraft that was damaged? The assessor/technician teams will need to contact an engineer that has training and skill in the repair of the currently damaged aircraft. There are many different approaches to this procedure, but the quickest seems by way of a computer communications system. ABDR assessor/technician teams would be able to contact an engineer through teleconferencing software when the on-base engineer is not skilled in the repair of the damaged aircraft. Since the assessor/technician teams will have PMA's that will be attached to the ABDR intranet, they will have the privilege in this case of being on-line giving the ability to contact someone through the net. This means that an engineer could be contacted through this same system.

**Hardware and software requirements for the demo:**

The demonstration of our research this summer will show the required capabilities of software that will enable the use of a communications system between assessors/technicians and engineers. The software for assessors and engineers in ABDR should include a software package that will allow for contact to other assessors and

engineers when needed. As said before, the need for an off-base engineer is only necessary when the on-base engineer is not skilled in the repair of the currently damaged aircraft. However, when these cases occur, communication for quick instruction is mandatory. The software or hardware for assessors and engineers should include the following or same functions as:

**-E-mail functions (Netscape or Lotus):** Standard E-mail capability is necessary in the transmission of important documents to the engineer from the assessor/technician. Using E-mail, you may affix files such as digital pictures and information that would best help explain the problem being addressed. The benefit of using Netscape is that this program allows plug-ins to be utilized.

**-Digital Camera/with software (Kodak):** Digital Cameras allow for pictures to be taken without film; being directly loaded on to the camera and then, downloaded on the computer. The software allows you to negate, soften, and modify pictures all on your computer. Software such as this allows usefulness to the user in that they can now modify areas they cannot make out in the normal picture.

**-Digital Video Camera:** Digital video cameras allow a person to take digital video footage without the use of film. For purposes concerning ABDR, these cameras will be of great importance being capable of feeding video into a computer and showing it to another individual across some kind of telecommunications software.

**-View Director Plug-in:** The View Director plug-in is a device that allows for several annotations to be made on pictures or files through the Netscape Navigator. This program allows also for messages or http://www.'s to be attached to hot-links on the picture, allowing for a more interactive explanation of the problem.

**-Software for teleconferencing (C U see-me):** Teleconferencing software is vital if the engineer doesn't understand the instructions given him by the assessor. Through the use of this software, the engineer and assessor/technician will be able to communicate in real time with voice and video. Video displays and audio capabilities provide users with a personal feel to the teleconference. Certain programs, such as C U See-Me ( provided by Cornell University), allow you a "chat room" to type in, amongst being allowed to have a teleconference with real-time audio and video.

During the use of this teleconference, the assessor of the aircraft can use a digital video camera to show the engineer the damage site(s) on the aircraft to make sure they understand the extent of the damage. The digital video camera provides an added depth to conferencing and communication because you now have a real-time visual link to help explain procedures. Along with the information being given to the engineer, the assessor should give a full account of his opinions on the damage so the engineer can get a feel for what's going on. Other software applications are available for use with teleconferencing programs such as in the one listed above, C U See-Me.

White boards are devices which add dimension to the conference. Electronic white boards add yet another visual link and are being integrated into various software programs designed for teleconferencing. White boards can be used in a variety of different ways such as: bringing up pictures to explain and/or annotate, share your workbook with another person in your conference, and transferring files to another user. White boards are useful workbooks that can be shared between two parties; benefiting both.

**-Encryption techniques (Simplicrypt):** Encryption is a technique which allows scrambling of computer codes to prevent unwanted entry into a system. The process of encryption can be integrated into encoding files and data that you wouldn't want to get intercepted by counterintelligence. Scrambling and unscrambling information through encryption requires some form of password. A system to encrypt in real-time during telecommunications would be something to look for in the future. When a teleconference is held, others could easily intercept the information being transmitted. Information such as ABDR cases would not want to fall into enemy hands, thus the need for a security device.

**-Microphone and speakers:**

Microphones and speakers provide an audible addition to the telecommunications software. Through this telecommunications software, using microphones and speakers, you can now talk in real time explaining in real-time the situation concerning ABDR.

Not everyone who is an assessor or engineer will need expensive hardware like digital cameras or microphones and speakers. Each depot or air base would most likely have a couple cameras on hand for assessors and engineers to check out of perhaps an "equipment library" since not everybody needs one 24 hours around the clock.

**Our Procedure and Demo for ABDR remote engineering request:** The procedure right now for requesting engineers to fix abnormal damages is to bring them to the remote location from the depot, sticking them in the thick of the action. This is very dangerous because of the vitality of the engineer. The following procedure is the work of both of the summer high school apprentices for AL/HRG the summer of 1997 to show the feasibility of a technological advancement in remote engineering requests.

**1) The Assessor Decides to Make a Remote Engineering Request:** When the assessor decides the damaged aircraft he is in charge of needs the assistance of an engineer, he, beforehand checks his technical order (T.O.) to make sure the aircraft is outside the repair instructions and beyond his knowledge of a repair job. The assessor chooses what engineer he would like to work with by way of whatever kind of aircraft it is, the type of damage it received, and other factors.

**2) Assessor Gathers Information to Send to the Engineer:** To accurately describe the information to the engineer, the assessor must provide enough data to him to make absolutely sure of his understanding. From the use of digital photographs taken at different angles, distances, and when compared with other objects around it, the photo acts as a guide for the engineer to know the extent of the damage. Besides the use of digital photographs, the assessor could also affix to a regular e-mail message any other piece of information he feels will help the engineer in his job. The e-mail message would have to be a written description of the damage received to the plane along with many other opinions and suggestions. The assessor would then send his e-mail message with the attachments to the engineer.

**3) The Engineer Receives and Examines the Information:** The engineer receives the information on the damaged aircraft from the engineer and examines what he

has given to him. If the engineer doesn't comprehend what the assessor is trying to tell him, the engineer can then call for a teleconference using computer software designed for such a purpose. Many software programs allow for real-time communication between two or more parties on your personal computer through voice, video, and written word. For our purposes in ABDR, however, the assessor can use the video capabilities to show the engineer the damage site on the aircraft. Another option with teleconferencing software is an electronic whiteboard which would serve as a means of communication through examples being drawn and annotations occurring on perhaps digital photos; all happening in real-time between assessors and engineers. With the use of teleconferencing software for your PC (engineer) and PMA (assessor), communication is now fast and accurate.

**4) The Engineer Makes a Plan for Repair:** The engineer, being at the depot, has the use of specific equipment he normally would not have if he were in a hostile environment. The engineer has the use of many publications on aircraft battle damage, the expertise on other engineers, and various other utilities not normally found in war-like situations. One of these tools used by the engineer, but not preferable by all, is Finite Element Analysis (FEA). FEA is used to test specific areas on a structure for stress, dynamics, and frequency, amongst other analysis that would test his plans for repair of the damaged aircraft. The assessor can then, after forming his test, make annotations to the assessors pictures that he was given him as well as making his own diagrams.

**5) The Engineer Returns Instructions for Repair:** After the engineer formulates his calculations and organizes information, he constructs an e-mail message with all attachments needed for explaining the solution for the battle damage problem. The engineer then returns e-mail to the assessor with the procedure for repair.

**6) Assessor Acquires the Repair Instructions from the Engineer:**

Once receiving the necessary instructions from the engineer, the assessor will put the repair into action. The assessor will read all the written instructions, view pictures, and make annotations; where need be. If the assessor still does not understand the engineers instructions, they can then call for another teleconference just like the option the engineer had earlier.

**7) Assessor Follows the Indicated Procedure:** Immediately following the assessor's understanding of the repair given to him by the engineer, the plane is fixed and the result is battle capable aircraft. By fixing the plane with the correct repair, the team chief pronounces the plane "battle ready".

## Conclusions and Suggestions for the Demo and Future of ABDR:

This project has allowed for me to see how technology can aid in ABDR. As new technology becomes available, I am sure people will utilize the functions and usefulness of some of this software. Some key issues on the security of this system would have to be considered. This topic brings up a subsidiary issue on encryption. As said earlier, encryption is a form of scrambling computer signals to deny access to specific programs, documents, etc. E-mail encryption would have to be looked into in detail because currently there are political arguments about it's legality and threat to national security. One strong argument that was made by my fellow high school apprentice, was that since the e-mail encryption would be for government use toward the cause of national security, the U.S. government may be able to get around this issue and use this type of program anyway. The real-time encryption problem is something that will have to be looked for in the future or developed. Real-time encryption will be applied to any form of telecommunications signals transferring back and forth between assessors and engineers.

Like with all technology, developers are always improving or making new software and hardware applications. It seems the philosophy I have found to be true in software is if you don't design it someone else will. Above all other things in this system for ABDR, acquire those hardware and software programs which are necessary to the system to make sure communication is fast, accurate, and safe.

# ALTERNATIVE TRAINING AGENTS
# LABORATORY-SCALE WORK

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
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August 1997

# ALTERNATIVE TRAINING AGENTS
# LABORATORY-SCALE WORK

Lauren M. Spencer
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**Abstract**

The overall objective of this effort is to develop one or more clean, low-toxicity chemical agents with decreased stratospheric ozone depletion potentials, also referred to as ODPs, to replace Halon 1211 in firefighter training. This report describes laboratory-scale tests, mostly cup burners, of candidate alternate firefighter training agents that have acceptable predicted toxicity's, ODPs, physical properties, and availability. Fire extinguishment test methodologies and results obtained from the testing of existing halons and potential stimulant training agents are summarized and documented.

# ALTERNATIVE TRAINING AGENTS
# LABORATORY-SCALE WORK

Lauren M. Spencer

## Introduction

The laboratory flame extinguishment test accepted by the NFPA is the cup burner test (Reference 2). The cup burner apparatus consists of a glass chimney containing a small metal cup (flame cup). The chimney contains extinguishing agent that enters through a small tube at the bottom of the chimney. Introduced air mixes with the agent through the glass beads within the bottom of the chimney. Measured volumetric flow rates of air and agent are used to calculate the agent percentage molar (volume) concentration required to extinguish the flame. Reported extinguishment concentrations of the existing Halons range from 2 to 4 percent. The goal of this research is to find a safer and cleaner alternative to a Halon for firefighting. To reach our goals, we tested many agents that are all ready in use. Then, we created many different types of blends and tested them.

## Previous apparatuses

In 1961, a small cup burner was developed by Creitz (Reference 1). The burner had a 5.4mm ID glass chimney. Halon 1001 (bromomethane), Halon 1301, and nitrogen were tested.

Imperial Chemical Industries (ICI) developed the inverted sintered burner in 1970. Halons 1301 and 1211 were tested using various fuels. This burner did not work well, and its use was discontinued. To replace the inverted sintered burner, ICI built a cup burner, larger than that of Crietz, in 1973. The ICI cup burner chimney had an ID of 85mm and a height of 530mm, and the flame cup had and ID of 28mm and a height of 230mm. Halons 1301 and 1211 were tested. These tests, conducted by Hirst and Booth, are considered the standard industry (NFPA) tests. (Reference 2)

That same year, Factory Mutual Research Corporation(FMRC) attempted to duplicate the ICI cup burner. The FMTC cup burner had a much larger chimney with an ID of 105mm and a height of 400mm. The flame cup had an ID of 28mm (the same as the ICI cup burner) and a height of 220mm. The volumetric air flows of the ICI and FMTC cup burners were similar; however, due to the much larger FMTC chimney ID, the velocity past the flame cup was about 60 percent lower, and poor results were obtained.

In 1974, FMRC modified their cup-burner apparatus that liquid fuels could be vaporized before entering the burner chimney. The fuel was vaporized by using a syringe pump to deliver the liquid fuel into a heating block at a constant rate. Halons 1211 and 1301 were tested using the vaporizer attached to the burner apparatus. Concentrations of these agents, higher than those needed for the ICI standard cup burner, were required to extinguish the flame.

In 1987, NMERI (New Mexico Engineering Research Institute) constructed a cup burner to study the fire suppression characteristics of Halon 2402. The full-scale NMERI cup burner has a chimney with an ID of 88mm and a height of 764mm, slightly taller than that of the ICI cup burner. The flame cup has an ID of 25mm, a height of 364mm, and runs up through the center of the chimney. The flame cup is connected to a clear plastic tube that passes through the chimney bottom to a side-arm flask filled with n-heptane. The flask sits on an adjustable platform for fuel level control. The bottom of the chimney contains glass beads. A small glass mixing chamber (for mixing of air and extinguishing agent) is connected to the bottom of the chimney through a stopper. The extinguishing agent enters the mixing chamber through a side arm.. Air is introduced into the mixing chamber via another side arm with an interior attachment that channels the air to the bottom of the chamber, thus assuring the complete mixing of air and agent. Liquid agents are introduced into the mixing chamber by a syringe pump. (See Figure 1) The agent side arm is fitted with septum for the syringe needle. Gaseous agents enter through flow meters and tubing. (See Figure 2) Volumetric low rates of air and agent are used to calculate the agent percentage molar concentration required to extinguish the flame. This has been designated the "full-scale cup burner." (Reference 1)

During the fall of 1988, testing of Halon alternatives was initiated. For chemicals available in small quantities, the volume required to obtain results from the full-scale cup burner was too great, and the cost of agent per test was high. Therefore, two scaled-down versions of the full-scale cup burner were designated and constructed. The first version, a 5/8-scale cup burner, having a 37.5 percent reduction in linear dimensions, has a chimney ID of 57mm and a height of 438mm. (The original design had been slightly modified to include four sized ports for a thermocouple and for easier access to the flame cup for ignition.) The flame cup, which passes through a port in the side of the chimney, is 13mm in diameter, 165mm above the mixing chamber, and is placed in the center of the chimney.

The second scaled-down cup burner has a 2/5 scale, corresponding to a 60 percent reduction in linear dimensions. It has a chimney with a diameter of 33mm and a height of 307mm. The flame cup enters the chimney through a side port and has an ID of 9mm. The flame cup, inserting to the center of the chimney, is 173mm above the mixing chamber.

Three scaled mixing chambers were initially designed and constructed, one for each cup-burner apparatus. After initial tests with all three chambers, it was decided to use the largest chamber of each of the cup burners. The bottom surface area of the smaller chambers was too small to allow for rapid and complete liquid agent vaporization.

## Background

Halon firefighting agents, like chlorofluorocarbons (CFCs) have the potential to deplete stratospheric ozone and accelerate global warming. The threat to the global environment is considered so serious that, in 1987, the Montreal Protocol, an international treaty, restricted production of both Halons and CFCs. By the year 2000, Halon production for "non essential uses" will be halted as a result of the Protocol's review at the London Meeting in June 1990.

The Primary attributions of Halons include cleanliness,low toxcity, chemically active agent. The Halons are used around aircraft engines, weapon systems, computer facilities, and museum vaults without serious residual damage. The four necessary requirements set for Halon alternatives make agent selection difficult. These requirements are cleanliness, low ODP, low toxicity, and effectiveness. Other factors such as agent cost, stability during storage, electrical conductivity, compatibility with engineering materials, and global warming potential (GWP) must, of course, also be considered.

Many parameters must be taken into consideration when evaluating fire extinguishants. It is important to provide as detailed a description of the fire scenario as possible in order to define the best agent and technique for suppression. The wide variety of possible fire scenarios has led to development of many types of extinguishment systems. Because every fire situation is unique, the best approach for fire protection is to anticipate the potential hazards and make available the appropriate extingishant types and methods. Adequate test methods are required for each fire suppression scenario in order to develop and evaluate appropriate agents. Generally, fires have been divided into the following four classes (Reference 1).

1) Class A: Fires in ordinary combustible materials, such as wood, cloth, paper, rubber, and many plastics.
2) Class B: Fires in flammable liquids, oils, grease, tars, oil-base paints, lacquers, and flammable gases.
3) Class C: Fires that involve energized electrical equipment where the nonconductivity of the extinguishant media is of importance. (When electrical equipment is de-energized, extinguishers for Class A or B fires may be used safely.)
4) Class D: Fires in combustible metals, such as magnesium, titanium, zirconium, sodium, lithium, and potassium.

## Extinguishment Considerations

Most, though not all, Halon applications can be designated as either total-flood or streaming (localized).

- Total-flood systems are of three types: fire extinguishment (computer or control room protection), inerting (explosion or fire prevention for a gas leak inside on enclosure), and explosion suppression. The basic components of a total-flood suppression system consist of a pressurized agent supply connected by piping to one or more discharge nozzles located within the enclosure to be protected (or inserted) and a hazard detection system. Upon detection of a fire or a hazardous condition, a control valve releases the agent though the piping to one or more discharge nozzles. For fire suppressing and for concentration, a control valve is built up within the enclosure. Halon 1301 is the agent of choice for most total-flood systems, because of its lower toxicity and high effectiveness.
- Streaming applications are of two types: fixed and portable. Fixed systems are found in aircraft hangar protection systems. The components are similar to those in total-flood systems. The difference is that the agent nozzles are directed toward potential fire locations rather than designed to uniformly fill the enclosure. Manual systems came in sixes ranging from one pound hand-held fire extinguishers to five hundred pound flight-line truck units, such as in the Air Force P-19 vehicles. In the United states and Canada, Halon 1211 is generally the agent of choice for streaming applications.

## Discussion of Problem

The objective of this effort was to develop and use laboratory test methodologies and equipment to determine the fire extinguishment characteristics of candidate Halon alternatives for firefighting training. This effort was also designed to screen candidates for future field testing. Standardization tests have been performed on the cup burners using seven compounds that have a wide range of chemical and physical characteristics. Methods have been developed to analyze fluid-flow characteristics within the cup burners and are being developed for the laboratory-scale discharge apparatus.

In choosing a Halon alternate, they must have these qualities, cleanliness, low ODP, low toxicity, and effectiveness. Some of the important considerations that are taken into account are, cost, stability on storage, compatibility with engineering materials, electrical conductivity, and global warming potential (GWP).

Though cup burners are excellent for estimating the effectiveness of an agent in total-flood applications, they are less useful in determining the fire extinguishing ability of agents applied locally by streaming. Previous testing performed at NMERI has shown that physical properties related to streaming, fire penetration, and fuel securing is at least as important as extinguishment concentration in determining the fire extinguishing capability of streaming agents. Accordingly, a special laboratory apparatus had been designed to measure the effectiveness of an agent applied by streaming.

## Rotameter Calibration

The cup burner apparatus was developed to measure the vapor phase performance of a chemical as a fire suppressant. Volumetric flow rates of air and agent are used to calculate the molar percent concentration of agent required for flame extinguishment. Different techniques and equipment are required for testing gaseous and liquid agents. Gaseous agents are generally directed through a flowmeter; liquid agents are metered with a syringe pump.

Accurate air and agent flow rates must be known to determine extinguishment concentrations. Calibrated rotameters are used to measure flow rates in the NMERI apparatuses. Calibrating the rotameters consisted of determining flow rate versus rotameter setting for each combination of gases and settings. This section discusses calibration of the rotameters used to measure flow rates of air and agent in the cup burner apparatuses. Calibrations of the agent rotameters for eight standardization compounds are also included.

The rotameters used in the cup burner testing consist of a glass tube with a vertical scale marked from 0 to 150. The tube contains a spherical float with a diameter such that it is free to move vertically. At any flow rate within the range of the meter, fluid (in this case, gaseous agent of air) entering the bottom of the tube caused the float to rise. As the fluid flow increases, the float rises higher in the tube. Flow rate, therefore, is indicated by the position of the float on the graduated scale etched in the glass tube. The scale does not indicate flow rate directly, but the readings can be converted to flow rates for a variety of fluids. This conversion is accomplished by calibrating known fluid flows to rotameter settings.

Several methods can be used to measure fluid flow accurately when calibrating rotameters (reference 1). In this discussion, the fluids measured are air and gaseous agents. The technique used to measure flow rates was a simple bubble flowmeter.

The following section presents a discussion of the calibration procedures used to measure the air flow rate through the cup burner apparatus configuration by moving the tubing that ran from the rotameter to the chimney so that it ran from the rotameter to the flowmeter. By doing so, we can obtain our measurements needed to finish our calculations.

A bubble flowmeter was used to measure the flow rate. A hose, attached to the bottom of the chimney, led to a water displacement apparatus to measure the flow out of the chimney. The rotameter data was calculated to obtain an accurate point to test the bubble flow. Once the point was obtained the rotameter

was set to the point and the bubble meter was set in motion. Because of the difficulty of analyzing the data of the bubble flow two people were needed to be present to get an accurate flow readying from the bubble meter. While one person watched the rotameters setting and made sure it remained stable, the other took the calculations from the bubble meter. By watching the bubbles rise up the bubble meter and timing how long it took for one bubble to rise from the bottom of the bubble meter to the 10ml level, the person could obtain an accurate reading. By repeating the test at least six times to determine the time was stable we could be sure our readings were accurate. Once an accurate reading was obtained, the readings were calculated to get the final calculations.

## Methodology

The overall task included developing laboratory test protocols in order to assure that test results could be compared accurately as potential Halon alternatives were developed (Reference 1). The tasks conducted to determine the fire suppression characteristics of potential Halon alternatives included the following:

1. Reduced-scale cup burners and a laboratory-scale discharge extinguishment (LSDE) apparatus to determine, respectively, flame exinguishment concentrations and fire extinguishment with a streaming application for candidate agents were designed and constructed.
2. The cup burner was standardized using seven compounds having a wide variety of characteristics, such as extinguishment concentration, boiling point, density, vapor pressure, molecular weight, ad physical state (liquid or gas).
3. Methods to analyze fluid flow characteristics for the laboratory cup burners and the LSDE apparatuses were developed. This task standardized flow rates and operating procedures (i.e., test methodologies).
4. Performance of the above laboratory tests using existing Halons and potential Halon alternatives was determined. The results were compared and analyzed.
5. Methodologies to determine comparable extinguishment effectiveness parameters for Halon alternatives were developed.

## Test Description

A full-scale cup burner was scaled down to 2/5 scale to increase test precision and accommodate small quantities of candidate Halon alternatives. Seven chemicals (Halon 1211, Halon 1301, HFC-227, HFC-236, CF3I, C6, and Fluothane) were used for standardization, calibration, and characterization of the cup burner. The fuel used was n-heptane. Methods were developed to analyze fluid-flow characteristics within the cup burner. Agents and Blends of Perfluo-2-Butene, 1-Bromopropane, and HFC-227 were applied to the fire, and the minimum agent flow rates required to extinguish the fires were determined.

## Testing Procedures

Gaseous and liquid agents required different flow delivery techniques to the cup burner mixing chamber.

Gaseous agents are those chemicals with boiling points below room temperature (25 C). Gas flows were controlled by a flowmeter (rotameter), which could be adjusted to within 10 mL/min.

Liquid flows were controlled by the syringe pump speed, which changed by approximately 1 to 2 mL/min between settings. Liquid agents are chemicals with boiling points above room temperature. Agents with boiling points above room temperature are designated as liquid agents. These compounds could not be tested as gas agents are tested, because liquid agents are tested using the syringe setup, these agents would drip from the needle tip with a set amount of pressure applied to the plunger. Then a heater would heat the agent creating a vapor which would put out the fire.

The 2/5-scale apparatus configuration was used for our testing. The apparatus was set up in an explosion-proof fume hood. Gaseous agents were directed through a flowmeter connected to the air inlet of the mixing chamber. The agents were mixed with air in the mixing chamber. Passage of the mixtures through the beads ensured homogeneity. Liquids were placed in a syringe on an Orion Model 355 syringe pump. Highly volatile liquids were placed in a 10-mL syringe with an attached valve and syringe needle. The syringe needle for the liquids and highly volatile liquids was passed through a septum on the agent inlet port of the mixing chamber. Liquid and highly volatile liquid agents were vaporized with a heater and passed through the glass beads, where complete mixing occurred. Past the beads, the vaporized mixture continued upward through the chimney past the flame cup, which supported the n-heptane flame.

After the apparatus configuration was set up in the hood, the fuel was placed in a separate funnel resting on a laboratory jack platform that could be raised or lowered. A hose leading to the flame cup tube was attached to the bottom of the separate funnel. The flame-cup tube passed through the second port of the 2/5-scale apparatus. The fuel level in the flame cup was maintained at the rim by raising or lowering the fuel reservoir flask.

To measure the chimney temperature, the thermocouple was inserted through a septum in the lower ports of the 2/5-scale cup burner.. An air compressor supplied the air flow. The air flow pressure entering the mixing chamber was maintained at 26 lb/in2 with a pressure regulator. An air flowmeter setting was chosen to provide a stable flame. This air flowmeter setting was normally at an average of 60%, corresponding to 5890 mL/min.

1. Gaseous agents were stored in cylinders fitted with regulators connected to the agent flowmeter. The flowmeter was connected to the mixing chamber with tubing. The septum required for liquids was removed. The gas agent flow meter setting was increased by five units every 15 seconds until exinguishment occurred to establish a starting point. The next test began at a setting five units below this starting point, which was increased by 1 unit every 15 seconds until extinguishment occurred. Four additional test data points were obtained to define the minimum extinguishment concentration for the agent. Flow rates for the gaseous standardization compounds were determined differently from those for the other gaseous compounds (nonstandard agents) tested in the cup burner. The flow rates for gaseous standardization compounds were determined by calibrating rotameters specifically for each agent as discussed previously. When testing the nonstandard gaseous agents, flow rates at extinguishment were determined of agents available for testing did not allow for complete rotameter calibration, which was performed, and the chimney temperatures, air flowmeter settings, and agent flowmeter settings were averaged. The bubble meter was then used to measure a flow rate for the average agent rotameter setting. The average of three bubble meter measurements was used to determine the final agent flow rate.
2. For liquid agents, the testing procedure for standardization compounds and nonstandard agents was the same. The syringe was filled with agent, the needle was passed through the septum, and the syringe pump speed setting was chosen, the syringe pump and extinguishment occurred. At extinguishment, volume versus time readings were taken to determine the flow rate of agent exiting the syringe. A total of five tests were performed to determine the minimum extinguishment concentration. There was no time to test any of the highly volatile liquid agents. If there were time, the flow rates would be controlled using the same conditions that were used to test the other liquid agents. Generally 10 or more tests would be required to determine the average minimum extinguishment concentration.

The flow rate for liquids and highly volatile liquid agents was determined by timing the agent flow as it entered the mixing chamber. The flow was controlled by a syringe pump for the liquids and a syringe/needle apparatus for the highly volatile liquids. The concentration calculation for liquids and highly volatile liquids is the same.

## Results

At present, the method of choice for laboratory-scale fire suppression testing is the cup burner test developed at ICI by Hirst and Booth. Because this test requires a relatively large amount of chemical, smaller versions (5/8-scale and 2/5-scale) of the cup burner were developed as part of the Air Force program to develop Halon alternate. The 2/5-scale cup burner results at NMERI are presented herein.

Six compounds and two types of blends were evaluated with the cup burner apparatus (See Chart 1). The cup burner test results indicate that the blends of the HFC-227ea & 1-Bromopropane, Perfluro-2-Butene & 1-Bromopropane, the HFC-236fa & 1-Bromopropane, and the Perfluo-2-Butene should have further testing and evaluations done in the near term.

It is expected that the weight, volume toxicity, and containment considerations for these neat agents and blends will meet the criteria for an alternate firefighting agent. Field-scale testing should be performed with several of these candidate agents in order to validate the correlation between lab and field testing. Ideally, if a correlation can be found, the time required and costs associated with testing and development of long-term highly effective suppressants will be greatly reduced.

## Conclusion

It has been noticed by NMERI that the reduction in the cup burner size from a large scale to 2/5-scale also reduces the variance in fire extinguishment test results. The smaller cup burners are a valid alternative to the full-scale cup burner and enable the testing of compounds available only in small quantities. The addition of side ports to the 5/8- and 2/5-scale models provided easier access to the flame cup, making the testing procedure much easier. A syringe pump facilitated the testing of liquid agents. A buret/needles apparatus allowed for the testing of highly volatile liquid agents. Cup burner testing measures the extinguishing capability of a chemical compared to that of baseline agents: Halon 2402 (for liquids) or Halon 1211 and Halon 1301 (for gases).

Seven materials (Halon 1211, Halon 1301, HFC-227fa, HFC-236ea, CF3I, C6, and Fluothane) have been used for standardization, calibration, and characterization of the lab's cup burner using n-heptane fuel. These materials were compared to the results of the NMERI testing and used as a baseline, so testing of new agents could be continued. Flow rates of gaseous agents were measured with both rotameters and bubble meters. The delivery of liquid agents was monitored with a syringe pump, and a special mixing chamber was used to ensure vaporization and mixing.

As part of this work, the mechanics of ancillary equipment and of gas flow throughout the cup burners were determined. Testing was performed over a broad range of agent and air flow rates to develop curves of extinguishment concentration versus total flow. Special care was taken to ensure calibration of all flow measuring equipment. In particular, rotameter calibration was essential to obtain accurate flow rates.

In summary, apparatuses permitting cup burner testing of reduced quantities of candidate agents and agent testing under streaming applications have been developed, standardized, and characterized. These

laboratory-scale tests allow the screening of large numbers of expensive and non-commercially available (non-bulk) chemicals before field testing, saving significant amounts of time and money. It is my opinion that our results were conclusive, but continuing the cup burner testing is crucial to the Halon alternatives program. There are many different blends that need to be created, studied and then tested before a final decision can be made.

1. Ted A. Moore, Joanne P. Moore, Jonathan S. Nimitz, Harold D. Beeson, and Robert E. Tapscott, Alternative Training Agents-Laboratory-scale Experimental Work, New Mexico Engineering Research Institute, University of New Mexico, Albequerque, New Mexico 87131.
2. Hirst, B., and Booth, K., "Measurement of Flame-Extinguishing Concentrations," Fire Technology, Vol. 13, No. 4, 1977.

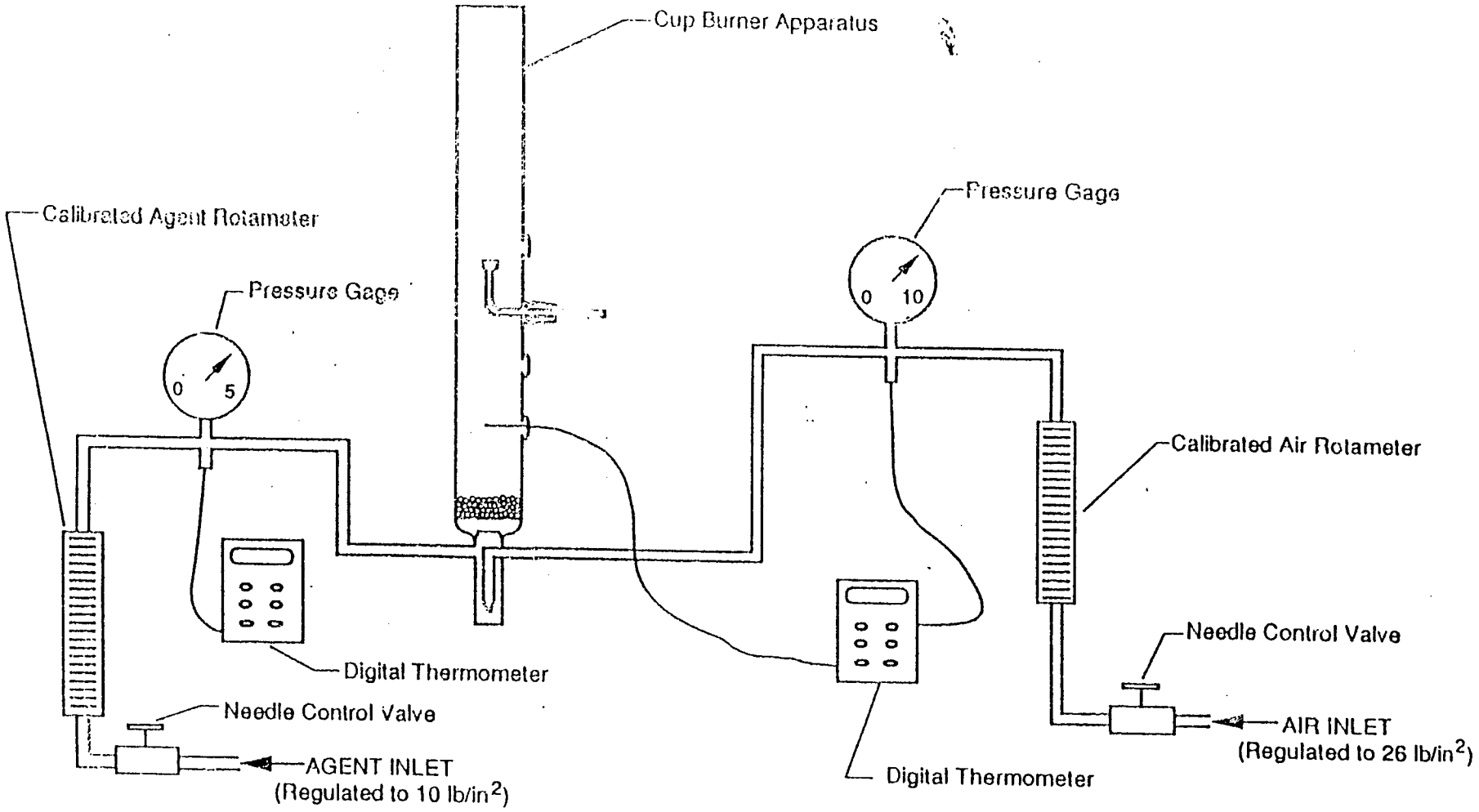



Figure 1; Typical Cup Burner Apparatus Configuration for Testing Gaseous Standardization Agents.

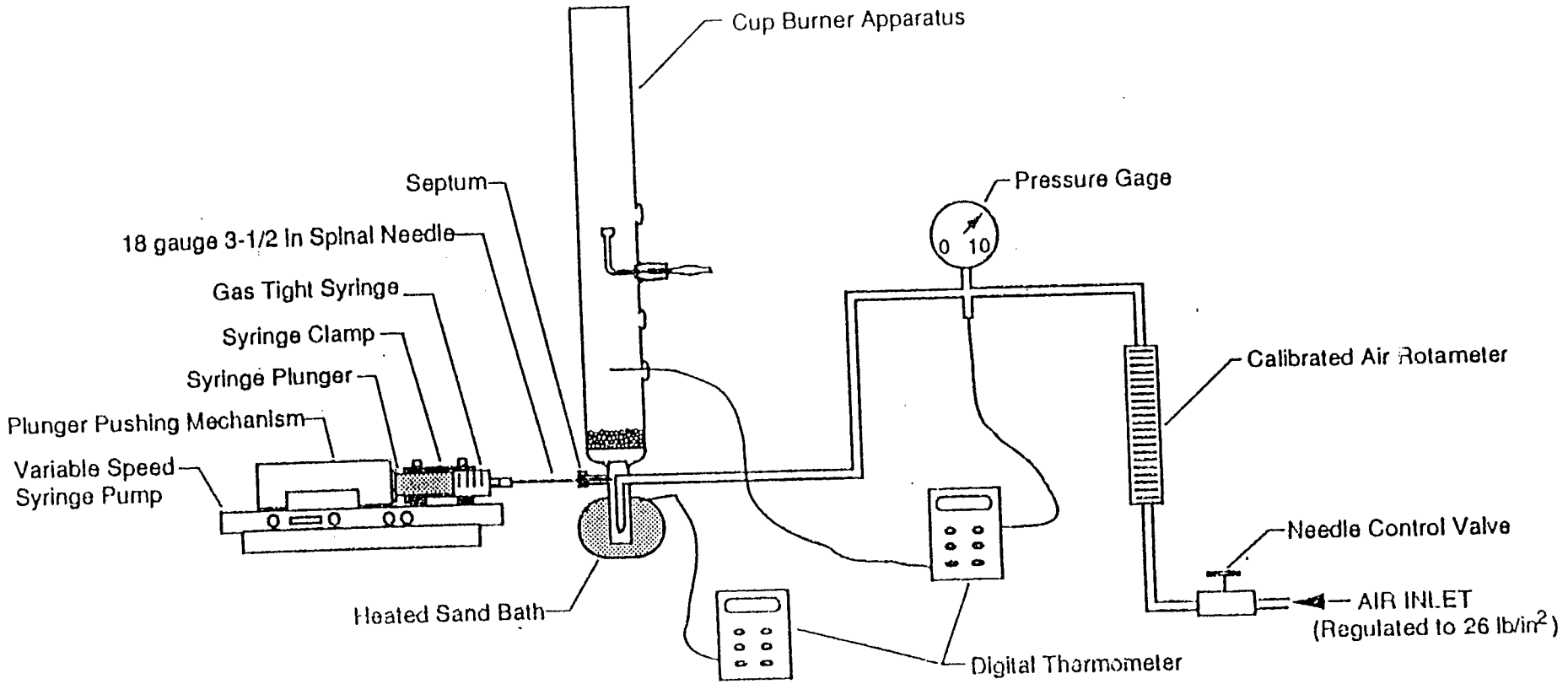



Figure 2; Typical Cup Burner Appartus Configuration for Testing Liquid Agents

Chart 1; Standardization Agents

## Gaseous Agent Extinguishment Concentration Calculation

| Standarization Agent | Experiment figures (Rotameter) | (Flowmeter) (seconds) | Flow Rate (ml/min) | Air Flow (ml/min) | Cup Burner Values (%) |
|---|---|---|---|---|---|
| CF3I | 65 | 3.23 | 186 | 5890 | 3.06 |
| HALON 1211 | 67 | 2.94 | 204 | 5890 | 3.35 |
| HALON 1301 | 58 | 3.59 | 167 | 5890 | 2.76 |
| HFC-227ea | 115 | 1.58 | 380 | 5890 | 6.06 |
| HFC-236fa | 123 | 1.50 | 400 | 5890 | 6.36 |

## Liquid Agent Extinguishment Concentration Calculation

Agents: C6

| Experimental Figures | | Derived | |
|---|---|---|---|
| Air Flowrate | 60 | 5890 | (ml/min) |
| Atmospheric Pressure | 30.03 | 1.00 | (atm) |
| Chimney Temperature | 102.9 | 375.9 | (Kelvin) |
| **Air Molar Flow Rate** | | 0.19 | (moles/min) |
| Agent Flowrate | 0.093 | 1.89 | (ml/min) |
| Agent Density | 1.68 | | |
| Agent Molecular Weight | 338.04 | | |
| **Agent Molar Flow Rate** | | 0.009 | (moles/min) |
| **Concentration** | | 4.67 | (%) |

Agents: FLUOTHANE

| Experimental Figures | | Derived | |
|---|---|---|---|
| Air Flowrate | 70 | 6870 | (ml/min) |
| Atmospheric Pressure | 30.03 | 1.00 | (atm) |
| Chimney Temperature | 93.5 | 366.5 | (Kelvin) |
| **Air Molar Flow Rate** | | 0.23 | (moles/min) |
| Agent Flowrate | 0.058 | 1.02 | (ml/min) |
| Agent Density | 1.87 | | |
| Agent Molecular Weight | 197.382 | | |
| **Agent Molar Flow Rate** | | 0.010 | (moles/min) |
| **Concentration** | | 4.03 | (%) |

## NMERI Extinguishment Concentration Calculations

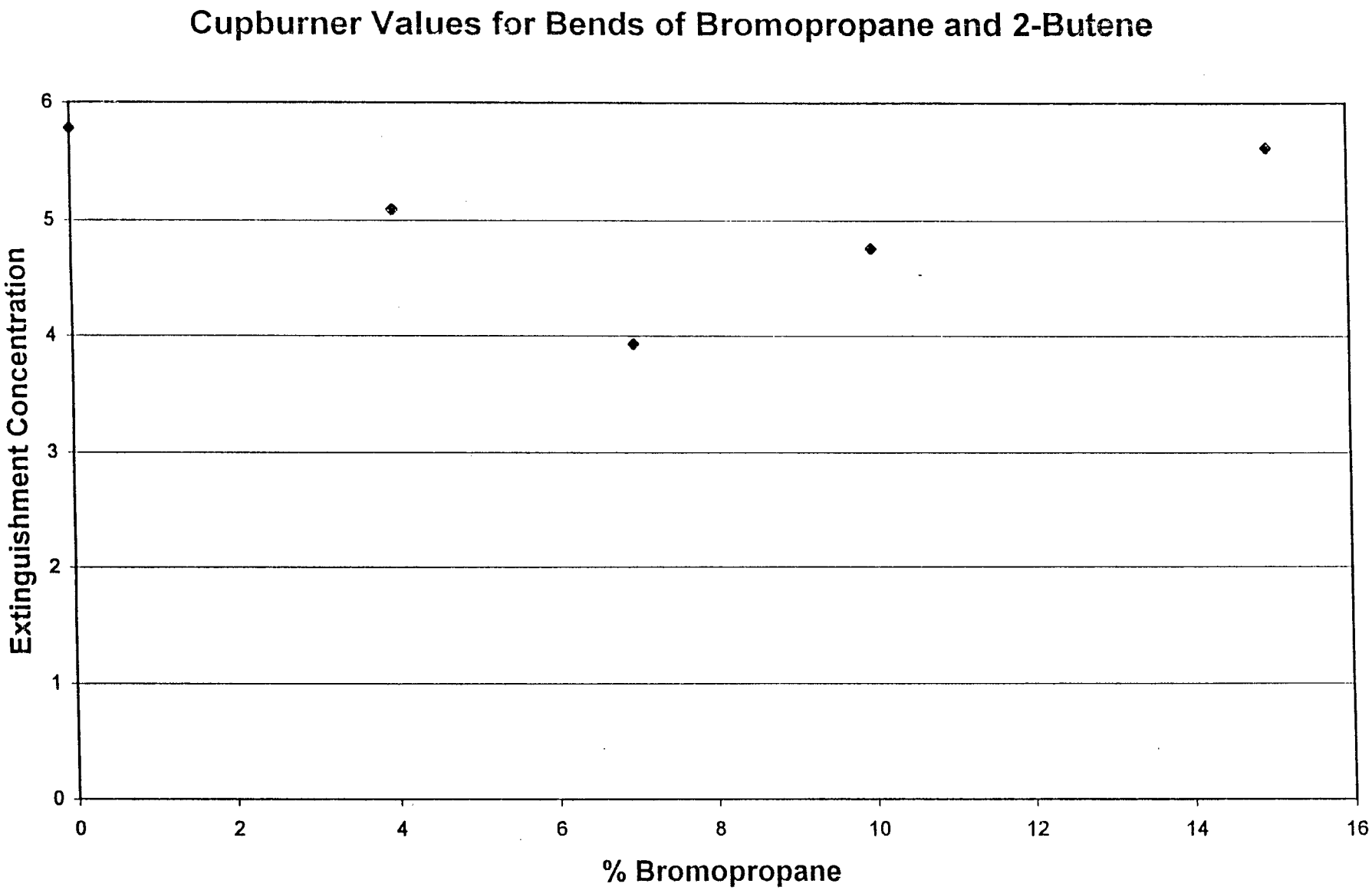
| Coumpound Name | NMERI Cup Burner Values (%) | % Differences (NMERI/ours) (%) |
|---|---|---|
| 1211 | 3.22 | 4.00 |
| 1301 | 2.90 | -4.85 |
| CF3I | 3.02 | 1.24 |
| C6 | 4.42 | 5.6 |
| HFC-236fa | 5.60 | 13.6 |
| HFC-227ea | 6.30 | -3.86 |
| FLUOTHANE | 3.12 | 29.2 |
| **Average Deviation=** | | 6.41 |

## Gaseous Agent Extinguishment Concentration Calculation

| Standarization Agent | Experiment figures (Rotameter) | (Flowmeter) (seconds) | Flow Rate (ml/min) | Air Flow (ml/min) | Cup Burner Values (%) |
|---|---|---|---|---|---|
| 2-BUTENE | 117 | 1.66 | 361 | 5890 | 5.78 |

## Blended Agent Extinguishment Concentration Calculation

| Blended Agents | Experiment figures (Rotameter) | (Flowmeter) (seconds) | Flow Rate (ml/min) | Air Flow (ml/min) | Cup Burner Values (%) |
|---|---|---|---|---|---|
| (96:4) 2-BUTENE & BROMOPROPANE | 105 | 1.88 | 319 | 5890 | 5.14 |
| (93:7) 2-BUTENE & BROMOPROPANE | 98.3 | 2.49 | 241 | 5890 | 3.93 |
| (90:10) 2-BUTENE & BROMOPROPANE | 98.3 | 2.04 | 294 | 5890 | 4.76 |
| (85:15) 2-BUTENE & BROMOPROPANE | 105 | 1.71 | 351 | 5890 | 5.62 |
| (95:5) HFC-236 & BROMOPROPANE | 102 | 1.80 | 333 | 5890 | 5.36 |
| (92:8) HFC-227 & BROMOPROPANE | 108 | 1.90 | 316 | 5890 | 5.09 |


Cupburner Values for Bends of Bromopropane and 2-Butene
Extinguishment Concentration
0
1
2
3
4
5
6
0
2
4
6
8
10
12
14
16
% Bromopropane

# A STUDY OF ACCURACY AND RESPONSE TIME IN TESTS OF SPATIAL ABILITY

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Final Report for:
High School Apprenticeship Program
Armstrong Laboratories

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# A STUDY OF ACCURACY AND RESPONSE TIME IN TESTS OF SPATIAL ABILITY

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## Abstract

An experiment designed to test spatial abilities of human observers was produced at Armstrong Labs. The experiments consisted of rotating decahedrons. The experiment was timed. The experiment placed certain variables on the subject throughout the different experiments, including angular difference, speed of rotation, and tilt angle. No results have as yet been formulated. Data will be used for the future enhancement of training. The experiment evaluated accuracy and the time required for subjects to make geometric judgments.

# A STUDY OF ACCURACY AND RESPONSE TIME IN TESTS OF SPATIAL ABILITY

Tyler W. Standage

## Introduction

The experiment was designed as a program on a computer. The subject was required to test themselves in the domain of spatial ability. All data was recorded and placed on a spread sheet which was used to produce graphs which would be used to interpret the results of the experiment. The rationale for the experiment was to find any patterns or functions that may describe the response times as well as the accuracy associated with these phenomena. The experiment was a program run on a computer. The computer images were generated and these were interpreted by the subject. The computer system used was specialized to run these three dimensional graphics. The **Assessment of Perception-Taking Ability Program (APTA)**is an interactive graphics application developed for Armstrong Laboratory. Psychologists from the Human Factors Division will use this software to study the ability of human observers to determine if the coloring of hidden faces of dodecahedra are equivalent to the coloring of the faces displayed to the subject. There are two dodecahedra visible on the screen. Each figure is an opaque dodecahedron; each face of each figure is a solid color. The experiment itself consists of a sequence of trials.

In each, the program presents the subject with a rotating dodecahedron on the left side of the console and a similar figure on the right. The experimental task requires that the subject if the display on the right is the same as or is the mirror image of the figure on the left. Once the viewer has decided, he/she clicks on the left mouse button if the figures are the same or the right mouse button if the figures are different, thereby ending the trial. During the trial, the subject can PAUSE the trial by depressing the center mouse button. The experimental data is stored in a file, RESdata, which can be analyzed independently of the APTA program. In addition to presenting experimental trials, the APTA software includes an experimenter interface to allow the investigator conducting the trials to specify the

experimental parameters interactively. The interface uses pull-down menus and Dialog boxes. It also incorporates pushbuttons, which generally allow you to initiate some action. They appear on the screen as three dimensional rectangles labeled with text which indicates a related action. The numbers and the angles of rotation of each figure relative to each other are under the control of the experimenter. For example, the dodecahedron on the left can be rotating at 30 degrees relative to the dodecahedron on the right, while both are rotating at a tilt of 60 degrees. This is specified through the experimenter interface.

Methodology

The experiments had two variables for each series. In experiment one, two dodecahedrons were placed on the screen. There were four colors which were placed three times each on various places surrounding the three dimensional figure. No face touches the face of another with the same color. The dodecahedrons spin on a specific, predetermined course, at a specific speed, with variable tilt and interval(the two variables). Before beginning the subject would enter the specifics of the block of data he will be doing. These include the variable tilt and interval arrangements. Then the subject is ready to begin collecting data. The computer creates the two images to either be the same image, or a mirrored image of the base figure. The subject is put through a series of these situations in which the subject must determine if the dodecahedrons are the same, or mirrored(different). The subject would push the left mouse button if the figures were the same and the right mouse button if the figures were different. A series would include about four hundred of these situations. The full series is called a trial. The subject will go through ten trials of this experiment. The full ten trials is referred to as a set. Then the color number was increased to twelve, with a different color on each face of the dodecahedron. The subject would then again set the specifics of his trial and begin. The same number of series/trials/sets were completed for the twelve color.. All of the variables remained the same for the twelve color as were used in the four color. After the subject has completed all the trials required, the data is then placed onto a spread sheet. Various columns on this sheet would include trial number, correct/incorrect responses, response times, mean response times, and so on. Various 'dummy' variables were also formulated so the equations would work for the other columns.

These excel files were then transferred to SPSS where various graphs were produced. These graphs showed mean times against tilt and interval. They also portrayed the response times to number correct, and so on.

In experiment two, the two variables were speed and tilt.. The speeds range from one to five. Five being the fastest and one the slowest. The speed was not changed during the trial, but however the speed was changed with the trial number. The subject in this experiment was not required to set the tilt as this variable was now a constant for this experiment. The subject did however need to set the interval as well as the speed. The subject went through the same number of series in a trial as well as the same number of trials per set, for both the four and the twelve color. After the experiment was completed the data was again moved to SPSS where various graph were produced. These include the response times as compared to speed as well as mean response times compared to speed.

In the third experiment the dodecahedrons were placed exactly 180 degrees opposite one another. There were no variables that needed to be set for this experiment so the subject could get the trial going immediately. For the twelve color there was a key at the bottom of the screen which showed which colors were opposite which. The four color set did not feasibly need a key as the subject could adequately compare the two figures and decide whether or not the objects were the same or different. One set was completed for both the four color as well as the twelve color. The data was again placed into graphs which mostly showed the response time as compared to the way the figures were placed.

In the fourth experiment the objects were different. They were changed to two dimensional figures. There were two basic figures which were placed on the screen at variable tilts. The subject must then describe the figures as being same or different. In this experiment there were only 192 series per trial.20 trials of this experiment were completed per subject, making two sets. No graphs have as yet been produced for the results of this experiment.

Results

No results have as yet been produced for these experiments. But an idea has been firmly

established. The results will be used to create better tests for weapons systems operators. Having these people run through these tests may assess their ability to mentally picture the environment around them. It may be that this can improve their spatial ability as well. Targets can be better tracked. More targets can be tracked with great precision. Pilots will be able to get a nose on the enemy before the enemy can do the same. The effects of this experiment also stem into the ground. The radar operators also must have a keen ability to pick out targets and know proper heading and altitude. The idea is to increase these people's situational awareness.

# THE PROCESS OF TECHNICAL PUBLICATION/DOCUMENTATION VIA ELECTRONIC MEDIA FOR THE ARMSTRONG LABORATORY

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
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and

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August 1997

# THE PROCESS OF TECHNICAL PUBLICATION/DOCUMENTATION VIA ELECTRONIC MEDIA FOR THE ARMSTRONG LABORATORY

Rachel Strickland
A.C. Mosley High School

## Abstract

The participation of working at Armstrong Laboratory has been a learning experience. The Programs and Plans Department supports the laboratory in the technical documentation part of the laboratory. Technical reports are an important part of the process of publication of the experiments conducted at Armstrong Laboratories. This department also does the marketing which is done with pamphlets and booklets distributed during conferences and seminars. Also, a photo file was established to help organize pictures what the scientists were engaged in with their experiments. The most educational part of the summer was in researching the Environmental Protection Agency 17 Industrial Toxins Project Chemicals. This is a list of health hazardous toxins was researched and a large of amount of useful information was found on the subject. This summer has been an experience that has helped to better understanding of what is offered in the work force. Many business skills have been learned that can help later in a business environment.

## THE PROCESS OF TECHNICAL PUBLICATION/DOCUMENTATION VIA ELECTRONIC MEDIA FOR THE ARMSTRONG LABORATORY

Participating in the High School Apprentice Program at the Armstrong Laboratory, Tyndall, Air Force Base, has been a rewarding as well as an educational experience. Working with the Programs and Plans Division of Armstrong Laboratory can help one understand the extensive technical aspects of the laboratory. This Division supports the laboratory in its mission of assessing and managing environmental risks associated with developing weapons systems and Air Force industrial operations. With this laboratory being the lead laboratory for environmental quality, it ensures readiness and maintains peacetime training missions and operations. The "value added" comes from reducing the environmental impacts and costs of ownership, and enhancing worldwide environmental quality. This Division also assists the laboratory as well as ensure all necessary documentation is done according to Air Force Standards.

Once research projects have been executed, the project managers produce technical reports, journal articles, briefings, etc. in order to inform other scientist and engineers as to what the projects were about and what was accomplished. The Programs and Plans Division's Technical Editors edit all the documentation to ensure all Air Force Policy and Standards were followed. These reports are submitted via disk as well as hard copy to a publishing company to be distributed. Once printed, they are returned to the Programs and Plans Division who then distributes them to the designated agencies, other Air Force Bases across the country, as well as the Defense Technical Information Center.

The Programs and Plans Division is also responsible for marketing the other Divisions of the Laboratory (i.e., Environmental Research Division, Environmental Risk Assessment Technologies Division, and the Environmental Risk Management Technologies

Division). The Environmental Research Division conducts fundamental research to meet Air Force environmental technology needs. The Environmental Risk Assessment Technologies Division provides technologies to model and assess the degree of environmental risks from Air Force mission-essential operations. They team up with laboratory scientist to assure the maximum results in developing results of basic research into a usable product. The Environmental Risk Management Technologies Division gives Air Force Commanders technologies to reduce or eliminate environmental risks that may impede Air Force readiness, training or peacetime operations. The Programs and Plans Division prepares pamphlets and booklets to assist the scientist and engineers promote as well as market their programs when attending a conferences, seminars, as well as symposiums.

To assist the other Divisions within Armstrong Laboratory a photo file was established. This entailed pictures taken of various technologies that the scientist were involved with.

A project that I was assigned to and found educational was to research the Environmental Protection Agency 17 Industrial Toxins Project Chemicals, or the EPA 17s. The research included finding out their molecular formulas, facts about the effects that these toxins have on humans and what the characteristics that each withheld. Each toxin researched caused some type of health problem (i.e., nausea, skin and eye irritation, leukemia, damage to major organs such as the lungs, kidneys, and liver and some even cause death).

The EPA 17 Chemicals were:

1. Benzene
2. Cadmium and Cadmium Compounds
3. Carbon Tetrachloride
4. Chloroform
5. Chromium and Chromium Compounds
6. Cyanide and Cyanide Compounds
7. Lead and Lead Compounds

8. Mercury and Mercury Compounds
9. Methylene Chloride
10. Methyl Ethyl Kettle
11. Methyl Isobutyl Kettle
12. Nickel and Nickel Compounds
13. Tetrachloroethylene
14. Toluene
15. 1,1,1-Trichloroethane
16. Trichloroethylene
17. Xylenes

These seventeen toxic chemicals are very important in meeting the waste reduction goals of the Air Force. They are 60% to 75% of VOC emissions, 60% to 80% of EPCRA chemicals, and 50% to 60% of hazardous waste. If the EPA 17 chemicals are eliminated, this will reduce the EPCRA chemicals, and the VOC emissions and hazardous waste. By reducing VOC emissions and hazardous waste chemicals, the Air Force will be better suited to meet the goals of pollution prevention.

Working at the Armstrong Laboratory, Tyndall, Air Force Base, has been an unforgettable experience. It taught me how to get along with others, how to work in a government office environment, as well as how to obtain the necessary skills that can be useful through out life, and an idea of what it is like to work in the real world . This job has also given me a good ideas of what I will choose as my career field. It has been an excellent opportunity to learn numerous items that were not known to me before. Many computer skills and the properly use of a business phone. It has been an unforgettable summer.

# ANAEROBIC DEGRADATION PRODUCTS OF TOLUENE AND LABORATORY MSDS MANAGEMENT

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Final Report for:
High School Apprentice Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
Bolling Air Force Base, DC

and

Armstrong Laboratory

August 1997

# ANAEROBIC DEGRADATION PRODUCTS OF TOLUENE AND LABORATORY MSDS MANAGEMENT

Lydia Ruth Strickland
A.C. Mosley High School

## Abstract

As a student in the High School Apprentice Program I found it to be a great learning experience. During the summer I organized the entire laboratories Material Safety Data Sheets into one master set. The six different labs contained their own sets of MSDS's and it was my responsibility to put them together. I assigned all 5,600 chemicals their own reference number for easier access to locate MSDS's. Also in my curriculum for the summer I tested contaminated groundwater to see if it contained a chemical called benzylsuccinic acid. This is a byproduct of organisms that eat the main ingredient in the jet fuel that contaminated the groundwater. I found this summers job to be a excellent opportunity for students like myself.

**ANAEROBIC DEGRADATION PRODUCTS OF TOLUENE AND LABORATORY MSDS MANAGEMENT**

This summer's experience of working as a High School Apprentice Student on Tyndall Air Force Base at Armstrong Laboratories has been an educational one. As a student I was assigned two tasks to complete during the nine weeks working here. With these two tasks, I was given the opportunity to learn important information about chemicals and the valuable learning experience of working in the laboratory. While in the laboratory, I learned many things such as: how to use tools, equipment, and conduct chemical tests using techniques I had never been exposed to before in chemistry. The first assignment was to organize the Material Safety Data Sheets (MSDS's) from six different laboratories into a efficient and easily accessible system. A Material Safety Data Sheet provides valuable information about handling hazardous chemicals. It informs one about what cautions should be used for each chemical. A MSDS also gives other useful information about the chemical, such as what form it is in, (liquid, solid, etc.), boiling and melting point. With the Air Force regulations there must be one for every hazardous chemical in the laboratory. This was a tedious and time consuming task to accomplish. I started with the largest laboratory's MSDS's list and ensured that data existed for all hazardous materials in the lab. After completing this task, I combined all of the MSDS's from every laboratory into one master list of Material Safety Data Sheets. This task was the most time consuming, taking four to five weeks to complete. To ensure the cleanliness and protection of the MSDS's, plastic sheet covers were placed on each sheet. Each chemical was assigned a reference number in alphabetical order; they

were numbered by tens so that the system could be updated as new chemicals were added to the laboratory. These numbers and corresponding chemical names were placed in a computer data base and on each Material Safety Data Sheet. Approximately 5,600 chemical's were processed. If it can not be found alphabetically in the book, then the person could simply type the name of the chemical in the computer and be given the reference number. Then one could be able to look it up more conveniently in the book of MSDS.

On Tyndall Air Force Base behind the flight lines in a vacant field there is a old fire training pit called FT-23. The fire training pit was used for training firemen to fight fires. They started the fires with a fuel called JP-4, and put the fires out with surfactant, or soap called Aqueous Film Forming Foams (AFFF). The training pit was used for approximately ten years, it has been out of use for about eleven years. As a result of the training operations, the fuel and AFFF has contaminated the groundwater at the site. Thirteen wells were placed at the site to monitor the contaminated groundwater. We went to the test site and took samples from all wells using a bottom discharge bailer. The sampling started with the cleanest wells and went to the most contaminated. We then put the water into liter bottles that were treated with 20mL of 6M HCl. The dissolved oxygen, pH , and temperature were taken with field calibrated probes.

This is where the scientists come into the picture. The wells are monitored every few months for water quality parameters, such as: pH, oxygen level, and temperature; as well as for chemicals in the jet fuel and AFFF. They were also tested to see if the contaminants are being naturally degraded. One of these tests is the one performed by me, with the help of some of the other scientists. We were looking for anaerobic metabolites of toluene, particularly benzylsuccinic acid. The reason we are looking for benzylsuccinic acid is when groundwater is contaminated the aerobic organisms in the

ground die and anaerobic organisms take over. When the anaerobic organisms take over they eat toluene, an ingredient in JP-4. After they eat the toluene they let off a byproduct called benzylsuccinic acid. Nothing likes to eat the benzylsuccinic acid so it is always there. It is not found normally in nature. The following are the steps that were taken. This method will separate all organic acids but we are primarily looking for Benzylsuccinic acid

1. Washed all glassware with 6 M of hydrochloric acid (HCl), which is a typical groundwater preservative for organic acid.

2. Put 1 mL of 4-Flurobenzoic acid (stock solution)as a internal standard. Spiked groundwater with 1 mL of Benzylsuccinic acid and internal standard This internal standard reacts like the benzylsuccinic acid and if it is not found at the end of the experiment then that is evidence of error.

3. Did an ether extraction. First time shake with 80 mL of ether and second and third times 40 mL ether. (separatory funnels)

4. Collect all of the ether together.

5. Combined ether extracts were placed in a Rotavapor to concentrate the Benzylsuccinic acid by evaporating the ether. The will allow us to work with a smaller, more concentrated substance. (heated water bath at 40° and pressure between 300-400 mm Hg)

6. Transferred concentrated substance to a 3 mL conical bottle. Put sodium sulfate in to get out water.

7. Add about 1 mL of diazomethane and add until solution stays bright yellow. The Diazomethane causes a chemical reaction with the organic acids and makes an ester.

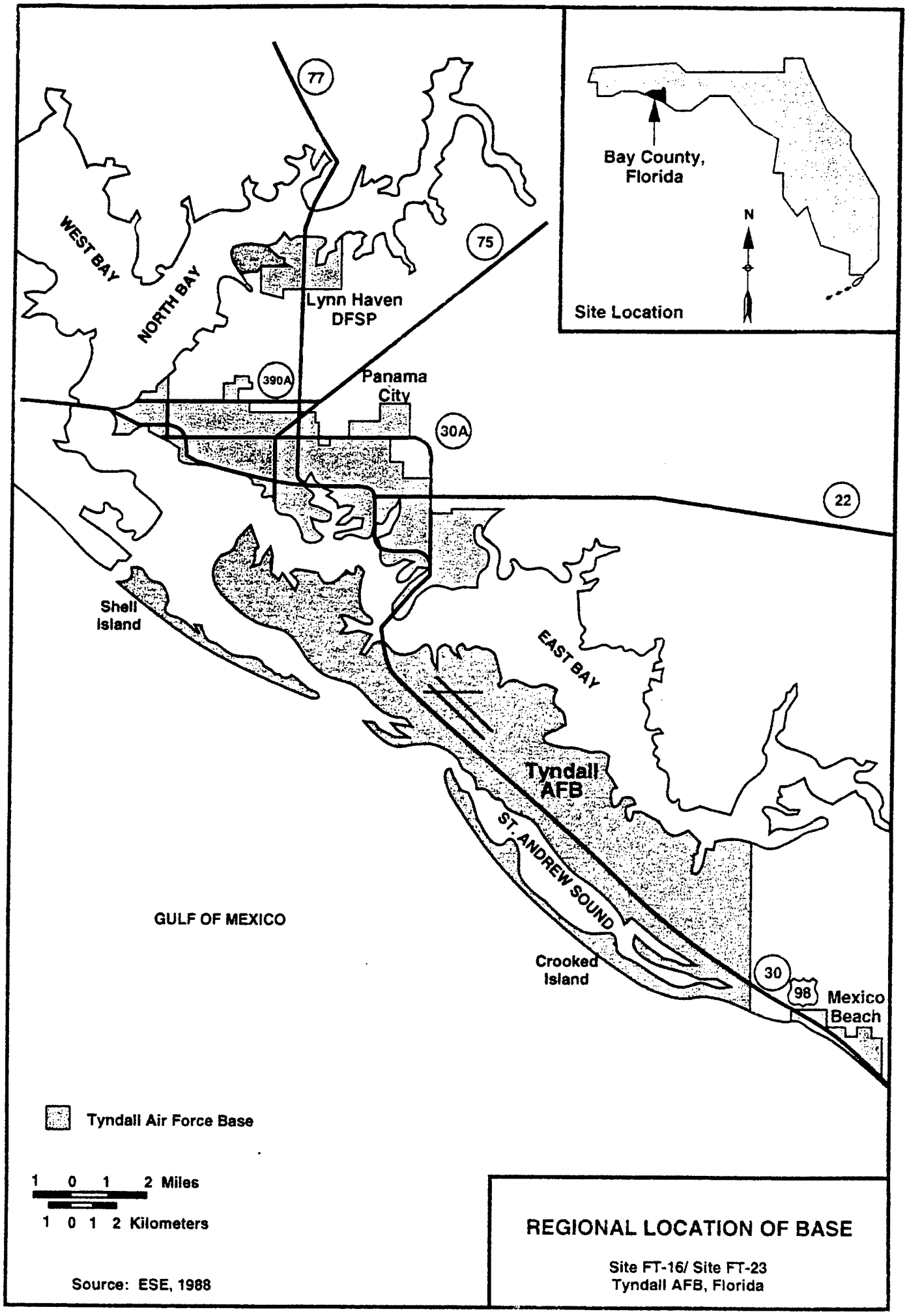
8. Put under helium blower to blow off the ether.

9. Redissolve in methylene chloride which is the solvent used for the gas chromatograph with Mass Selective Detector.

In our experiments we found no benzylsuccinic acid from the samples at the fire training pit. Because of this we decided to conduct another experiment. In the next

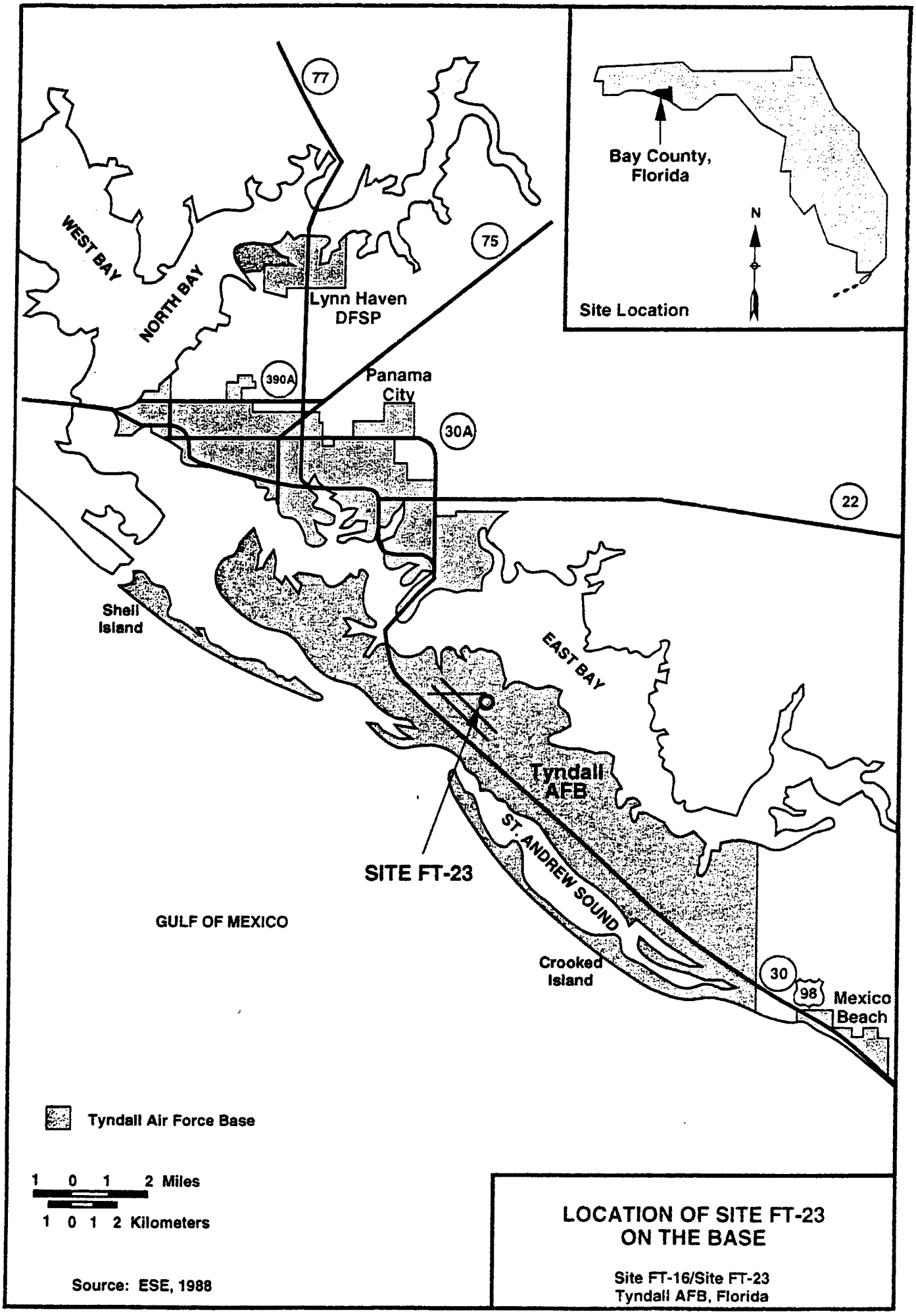
experiment we took distilled water and spiked it with benzylsuccinic acid in order to determine the detection limit for finding the benzylsuccinic acid. The first was 500mL of water with 0.25mL with the benzylsuccinic acid and that was undetectable. The same process was repeated with 0.5mL , 1 mL, and 2mL and peaks were seen in each. This enabled us to determine how little benzylsuccinic acid that could exist and still be detected.

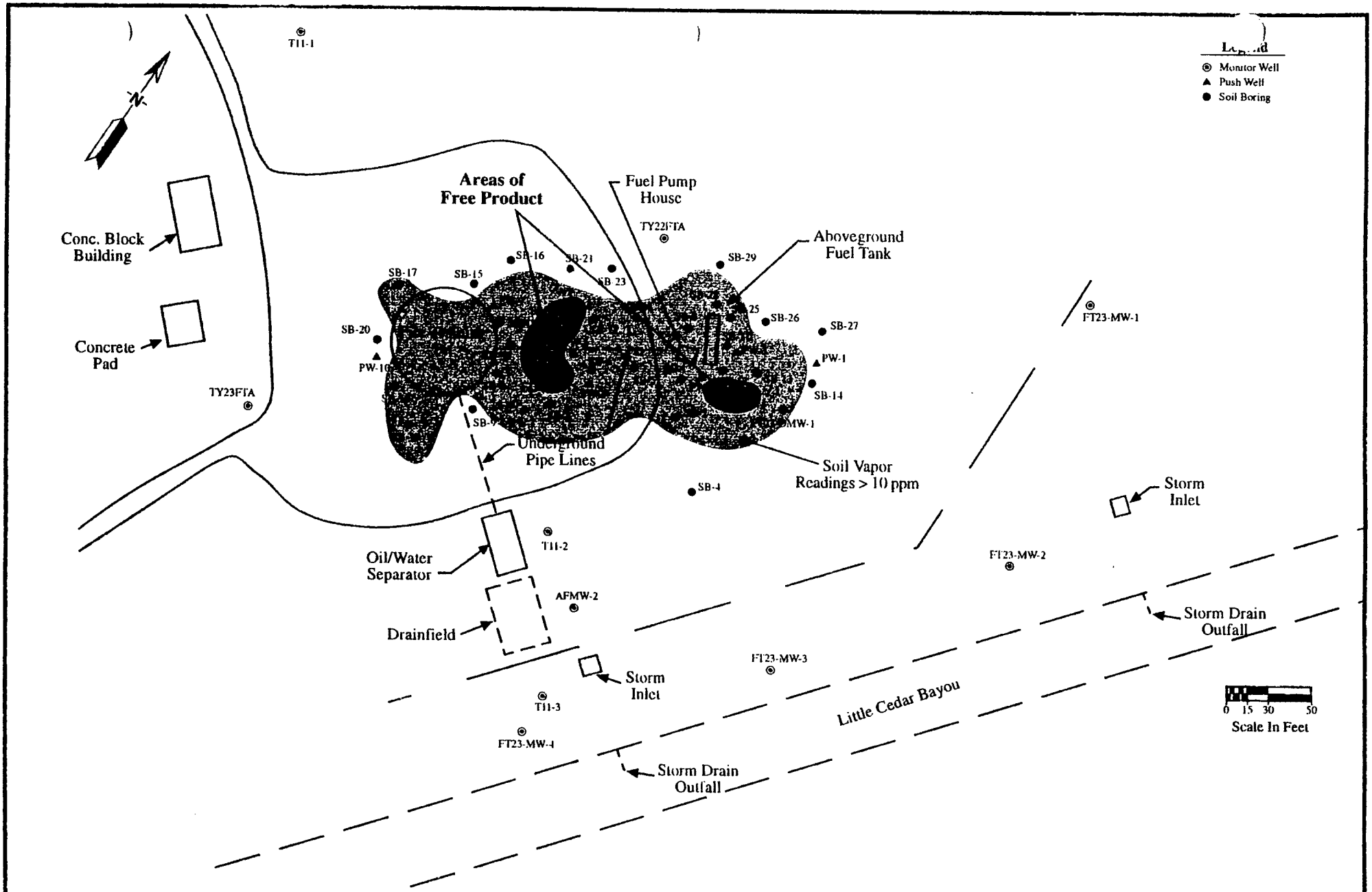
In conclusion my summer at Armstrong Laboratories has been the most educational and fun one yet. I am grateful that I have been able to work in this kind of learning, work environment that most of my peers are never fortunate enough to have. The skills that I have obtained working in the laboratory can be useful in the rest of my education and maybe even in my career. I hope to have the opportunity to do this again .



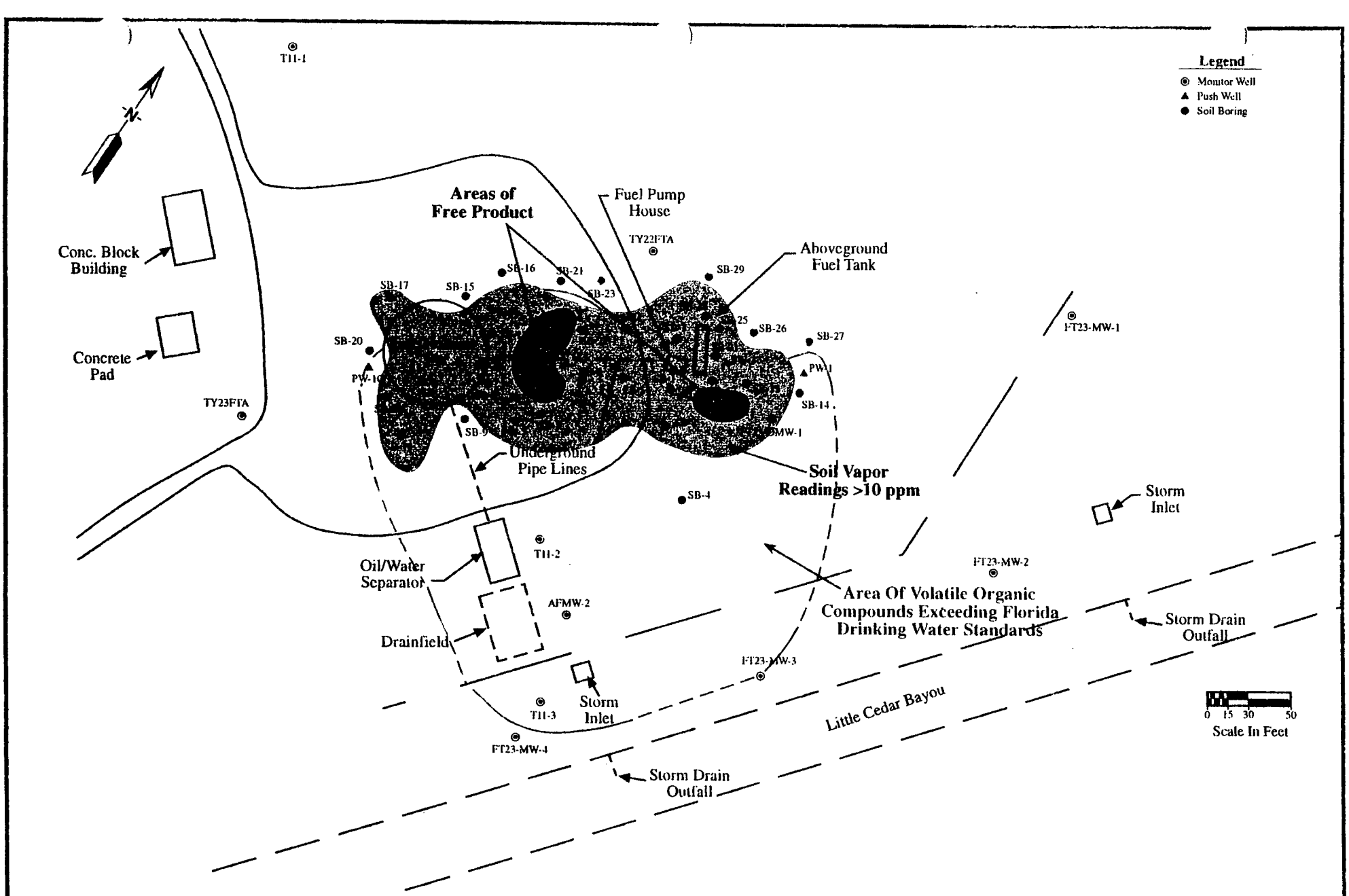

## REGIONAL LOCATION OF BASE

Site FT-16/ Site FT-23
Tyndall AFB, Florida


77
75
WEST BAY
NORTH BAY
Lynn Haven
DFSP
390A
Panama
City
30A
22
Shell
Island
EAST BAY
Tyndall
AFB
ST. ANDREW SOUND
SITE FT-23
GULF OF MEXICO
Crooked
Island
30
98
Mexico
Beach
Bay County,
Florida
N
Site Location
Tyndall Air Force Base
1 0 1 2 Miles
1 0 1 2 Kilometers
Source: ESE, 1988
LOCATION OF SITE FT-23
ON THE BASE
Site FT-16/Site FT-23
Tyndall AFB, Florida




**SITE FT-23 – AREAS OF FREE PRODUCT/EXCESSIVE SOIL VAPOR READINGS**
Site FT-16/Site FT-23
Tyndall AFB, Florida

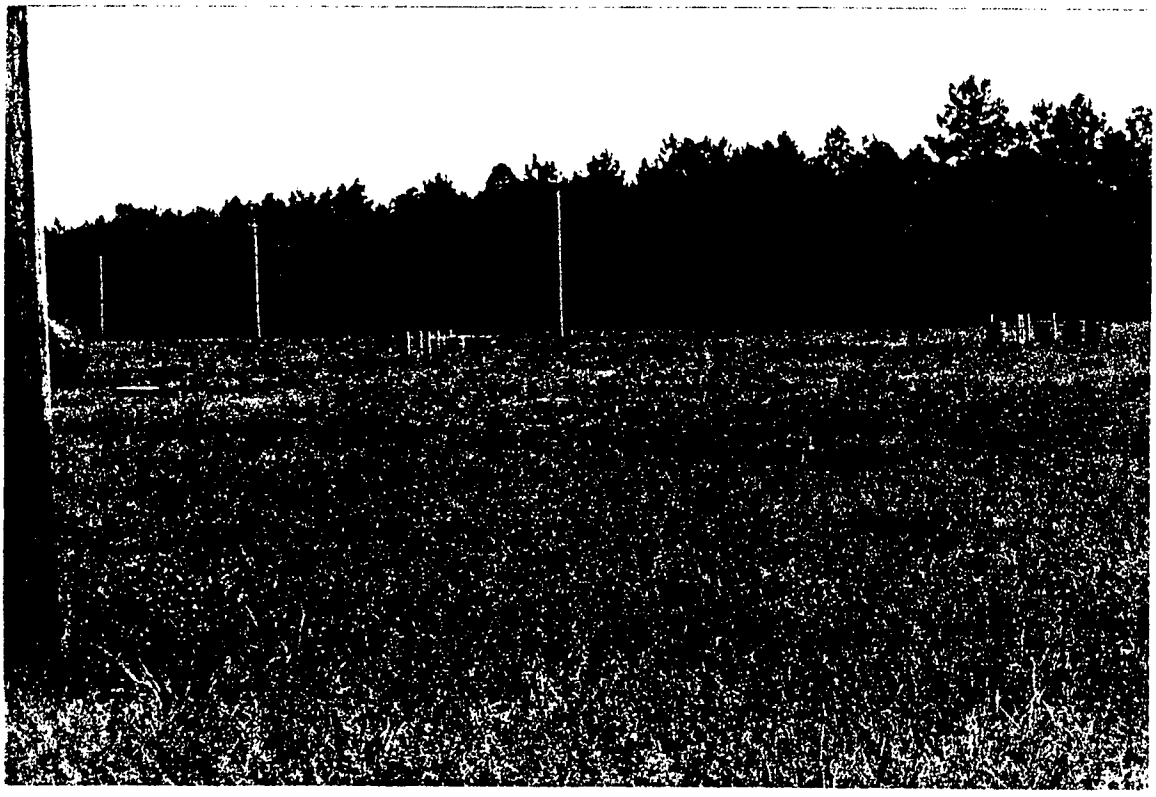



35-10

## SITE FT-23 - APPROXIMATE AREA OF GROUNDWATER CONTAMINATION

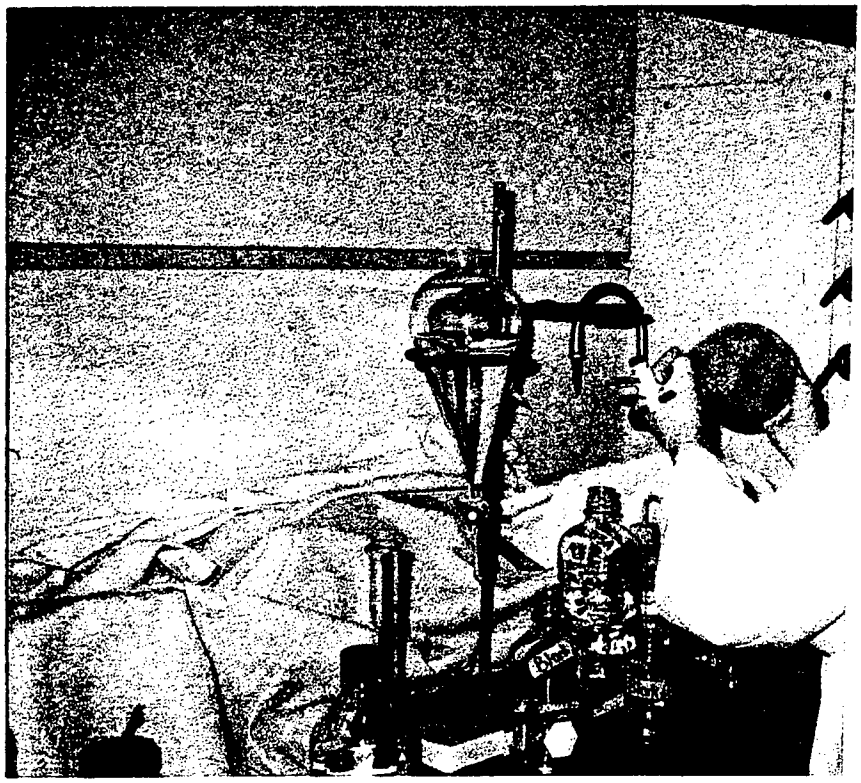
Site FT-16/Site FT-23
Tyndall AFB, Florida

This is an example of the wells at the fire training pit. It also shows the bottom discharge bailer.

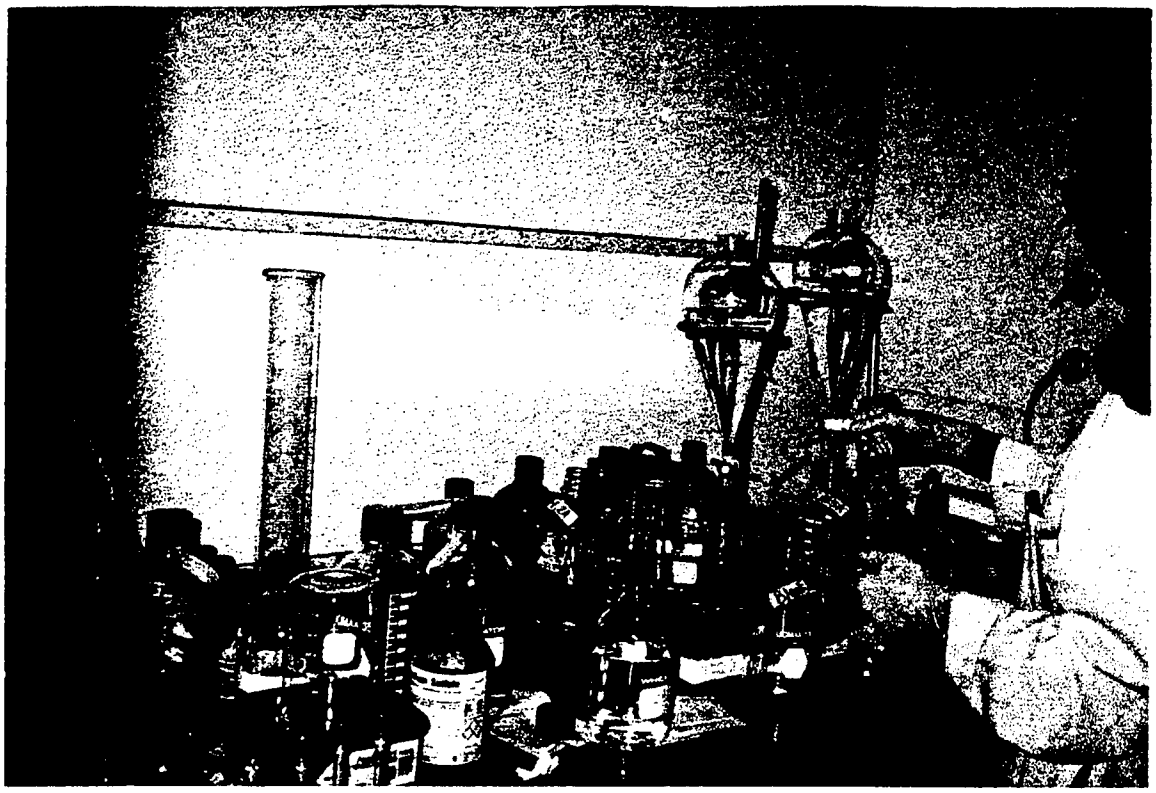


The fire traing pit was a grassy, vacant field as shown here. This shows two of the groundwater wells that the shamples were taken from.

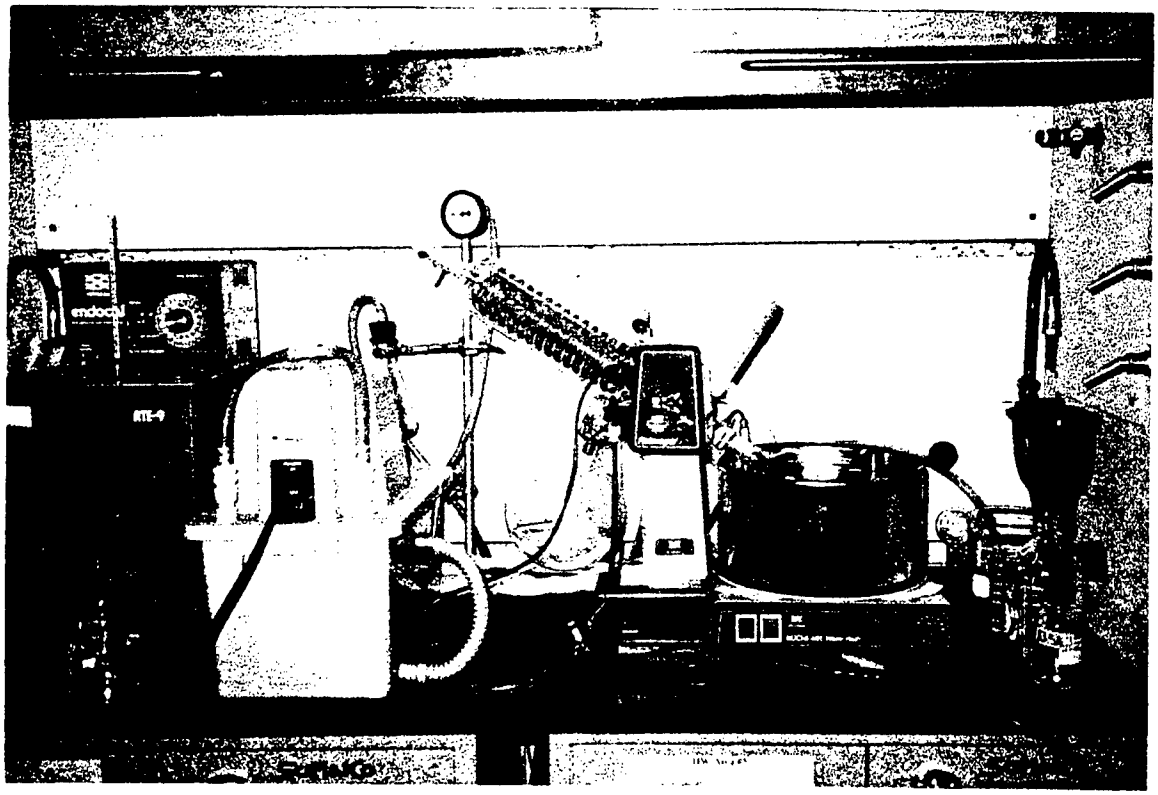
This picture shows the ether extractions as spoken of in number three of the procedure. Here the groundwater is being poured into separatory funnel.



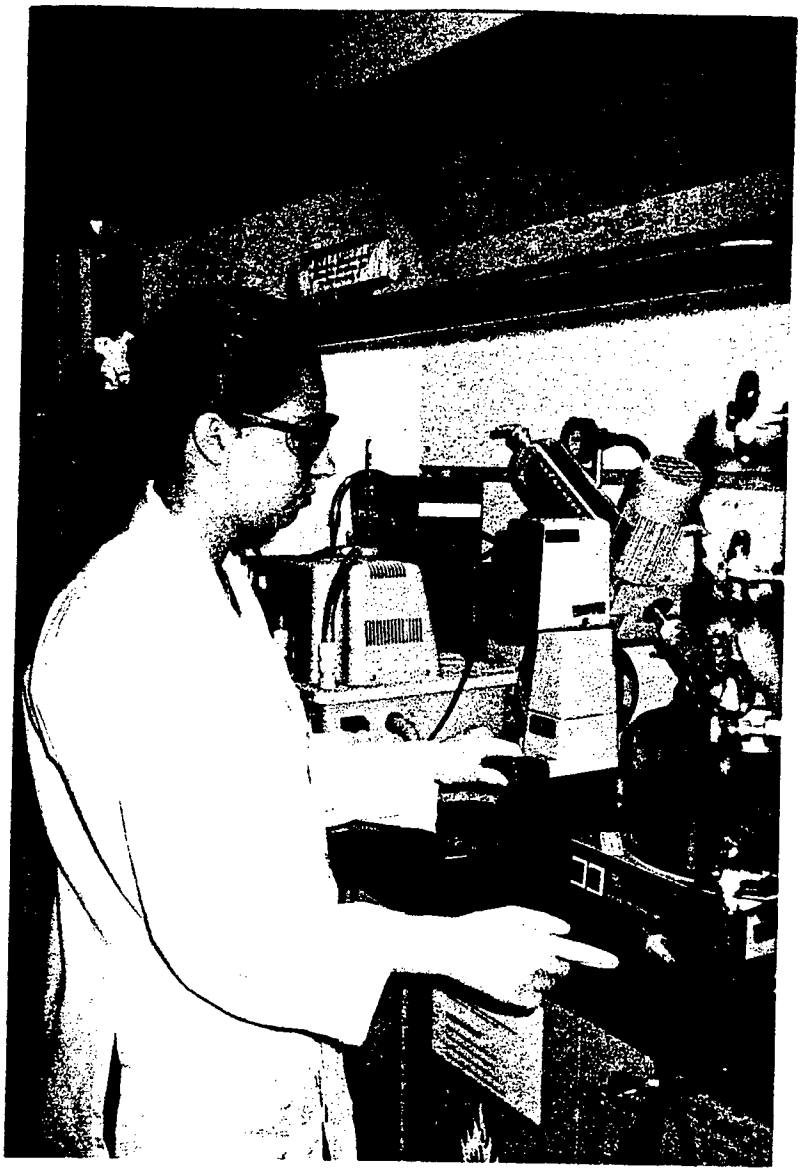
After the water is poured in the separatory funnel it is shaken up.



The water is then drained out and the ether is collected.



This is the Rotavapor which is used in step five of the procedure.



The ether in placed into the round bottom flask and lowered into the warm bath.

Bibliography

Field Sampling Plan; Rust Environment & Infrestructure.
Greenville, South Carolina; Version 2.0, April 1997

# The Effect of Hyperbaric Oxygenation on the Mitotic Division of Prostate Cancer Cells

Kelly Todd

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Final Report for:
High School Apprenticeship Program
Armstrong Laboratory

Sponsored by:
Air Force Office of Scientific Research
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and

Armstrong Laboratory

August 1997

# THE EFFECT OF HYPERBARIC OXYGENATION ON THE MITOTIC DIVISION OF PROSTATE CANCER CELLS

Kelly Todd
Theodore Roosevelt High School

Abstract

Prostate cancer is the most commonly diagnosed malignancy behind skin cancer. It is also the second leading cause of cancer death in U.S. men. Prostate cancer in its advanced stages is currently untreatable, thus there is a critical need for an effective treatment. For the experiment, malignant PC-3 cells were treated with hyperbaric oxygenation. It was hypothesized that the hyperbaric oxygen exposure will slow the growth rate of the cancer cells by stalling mitotic division. Effects of various pressure treatments ranging from 6 ATM to 0.32 ATM were tested. Experimental results indicated that .32 ATM was the most effective.

# THE EFFECTS OF HYPERBARIC OXYGENATION ON THE MITOTIC DIVISION OF PROSTATE CANCER CELLS

Kelly Todd

## Introduction

Hyperbaric oxygen is the inclusion of 100 percent oxygen at two to three times the atmospheric pressure at sea level. The resulting effect can include arterial oxygen tension in excess of 2000 mm Hg and oxygen tension in tissue of almost 400 mm Hg. The benefits of such oxygen doses include positive biochemical, cellular, and physiologic effects. The pressure expressed for hyperbaric oxygen is in multiples of the atmospheric pressure at sea level, which is 1 atmosphere. At sea level, the blood oxygen concentration is .3 mL per deciliter. Under the administration of 100 percent oxygen at ambient pressure increases the amount of oxygen dissolved in the blood fivefold to 1.5 mL per deciliter.

## Methodology

*Plating Cells:*

Prostate cancer cells were grown in flasks in RPMI-1640 growth media. For experimentation, the PC-3 cells were plated at 10^6 cells per plate in petri dishes. The plating of the cells was accomplished by decanting the media in the flask and adding a 2 mL wash of Trypsin-EDTA. This wash was decanted and then an additional 2 mL of Trypsin-EDTA was added. The flask was then incubated for 5 minutes at 37 C. After incubation the flasks were agitated to dislodge cells from the flask wall and 3 mL of RPMI-1640 media was added. The 5 mL cell solution was then pipetted vigorously to prevent cell clumping. After performing cell counts, cells were diluted to be plated in petri dishes at 10^6 cells per plate in 10 mL of media. The plates were then allowed to incubate overnight to ensure the binding of cells to the culture dish.

*Dosing Cells:*

After overnight incubation, plates to be dosed were decanted and 2 mL fresh media was added immediately prior to hyperbaric oxygen (HBO) exposure. Plates were then placed in the HBO chamber on a level surface to ensure even distribution of media/oxygen. The chamber was flushed at 10 ft. below sea level for 5 minutes. The pressure was then raised to the desired level at a rate of 1 ft/sec. Once the desired pressure was reached, the dosing continued for 1.5 hours. After the treatment period, the pressure was lowered at a rate of 1ft/sec to bring plates back to surface pressure. Plates then received an additional 8 mL of media and were returned to the incubator.

*Fixation of Cells:*

Plates remained in incubation for 23.5 hours. They were then spiked with 25 uL of 40 mM BrdU solution. They were then allowed to incubate 30 minutes. The cells were then harvested, saving all solutions in a 15 mL centrifuge tube. The media in the plates was decanted and a 1 mL wash of Trypsin-EDTA was added. This wash was decanted and an additional 1 mL of Trypsin-EDTA was added. The plates were then incubated for 10 minutes at 37 C. After incubation the cell solution was pipetted 30 times to break up and clusters and was then added to the 15 mL centrifuge tube. The plates were then washed with 1 mL of media two times, adding the remaining solution to the centrifuge tubes after each wash. The centrifuge tubes were then spun in the centrifuge at 1 K for 10 minutes. The supernatant was decanted and the pellet was resuspended in 1 mL of 70% EtOH over a period of 5 seconds while vortexing at the lowest possible speed. The fixed cell solutions were immediately placed in 4 C refrigeration and remained there overnight.

*Staining of Cells:*

The refrigerated cell solutions were spun in the centrifuge at 1 K for 10 minutes. After decanting the supernatant, the pellet was resuspended in 1 mL of PBTB. Each cell solution was then split into 2 microcentrifuge tubes (mct) to be stained. One mct of fixed cells received both Anti-BrdU and PI staining. The solution was centrifuged at 4 K for 8 minutes. The supernatant was decanted and the pellet was

resuspended in 1 mL of 2N HCl and then was incubated at 37 C for 20 minutes. The solution was then spun at 4 K for 8 minutes, decanted, and resuspended in 1 mL of 0.1 M Sodium tetraborate. The solution was allowed to incubate at room temperature for 5 minutes, was then spun at 4 K for 8 minutes, decanted, and resuspended in l mL of PBTB. This solution was immediately spun down at the above speed and time. After decanting the supernatant, the pellet was suspended in 20 uL of anti-BrdU FITC conjugated antibody and incubated at 37 C for 30 minutes. After incubation, 1 mL PBTB was added to the mct. The cell solution was then spiked with PI solution (final concentration 50 ug/mL) and RNAase (final concentration 10 ug/mL) and incubated at 37 C for 30 minutes, covered in foil. Following incubation, the stained cells were placed in 4 C refrigeration for no less than 30 minutes. The second mct of cells suspended in 500 uL of PBTB were stained with only the PI and RNAase in the manner described above.

Results:

The results of our experiment are as follows:

| **Cell Type** | **Cell Count** | | | | **Total** |
|---|---|---|---|---|---|
| Control PC-3 | 386 | 210 | 178 | 159 | |
| | 200 | 259 | 239 | 176 | 1807 |
| .32 ATM | 83 | 66 | 118 | 96 | |
| | 80 | 172 | 150 | 90 | 855 |
| .75 ATM | 133 | 144 | 161 | 120 | |
| | 133 | 126 | 139 | 126 | 1082 |
| 1.5 ATM | 145 | 142 | 200 | 150 | |
| | 152 | 115 | 118 | 105 | 1127 |
| 3 ATM | 101 | 106 | 115 | 146 | |
| | 118 | 138 | 145 | 241 | 1110 |
| 6 ATM | 120 | 200 | 108 | 111 | |
| | 93 | 104 | 93 | 103 | 939 |

Conclusion:

Based on the data collected, it can be concluded that hyperbaric-oxygenation can be used as an effective treatment for prostate cancer because Treatment would be most successful in stalling mitotic division at pressures of 0.32 ATM and 6 ATM. At .32 ATM, the number of cells decreased by 52.7% while at 6 ATM, the number of cells decreased by 48%.

# CEREBRAL HEMODYNAMIC RESPONSE TO A SQUAT-STAND AT 1G

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Final Report for:
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# CEREBRAL HEMODYNAMIC RESPONSE TO A SQUAT-STAND AT 1G

Tammy L. Venema
Walter E. Stebbins High School

## Abstract

Introduction: The squat-stand test was used as an orthostatic tolerance test by simulating the physiological effects an individual would experience in a high-G environment. A cerebral oximeter was used to test the hypothesis that the cerebral oxygen saturation and relative cerebral blood volume would decrease significantly when moving from a squatting to standing position. Methods: The subjects were instrumented with a cerebral oximeter and then asked to squat for four minutes followed by a two-minute stand. The cerebral oximeter used near-infrared light to measure the difference in specific wavelengths of the absorption of oxygenated and deoxygenated blood. A formula was then used to calculate the relative cerebral blood volume changes experienced. Results: The results indicated that an insignificant relative cerebral blood volume change occurred when the subjects moved from the squatting to standing position. Conclusions: The females in this study were capable of making physiological adaptations so that the loss of cerebral blood volume was insignificant.

# CEREBRAL HEMODYNAMIC RESPONSE TO A SQUAT-STAND AT 1G

Tammy L. Venema

## Introduction

Recent data reported form several investigations provides evidence that females have lower tolerance to various orthostatic challenges compared to males (1, 2, 3), and that these differences are associated with differences in blood pressure regulation in females (4, 5). If maintenance of arterial blood pressure is closely related to cerebral perfusions and consciousness, it is reasonable to predict that individuals with greater capacity to defend their blood pressure under high G challenges will perform better in combat conditions. Lower orthostatic capacity could clearly compromise the performance of the female warrior in a high performance aircraft (6). With this in mind, the squat-stand was used as an orthostatic tolerance test by simulating the physiological effects an individual would experience in a high- G environment. In the past, various orthostatic tests have been developed including: bedrest, to model cardiovascular changes that are associated with space flight; stand tests, where the subject is placed in a supine then standing position (7); endurance tests, performed by astronauts on landing day after spaceflight (8); and the standing of subjects before and after a VO2max test (9). In our study, a cerebral oximeter was used to record the cerebral regional oxygen saturation and relative cerebral blood volume when moving from a squatting to standing position. Hypothesis: A significant decrease in relative cerebral blood volume will occur when the subject moves from the squatting to standing position.

## Methodology

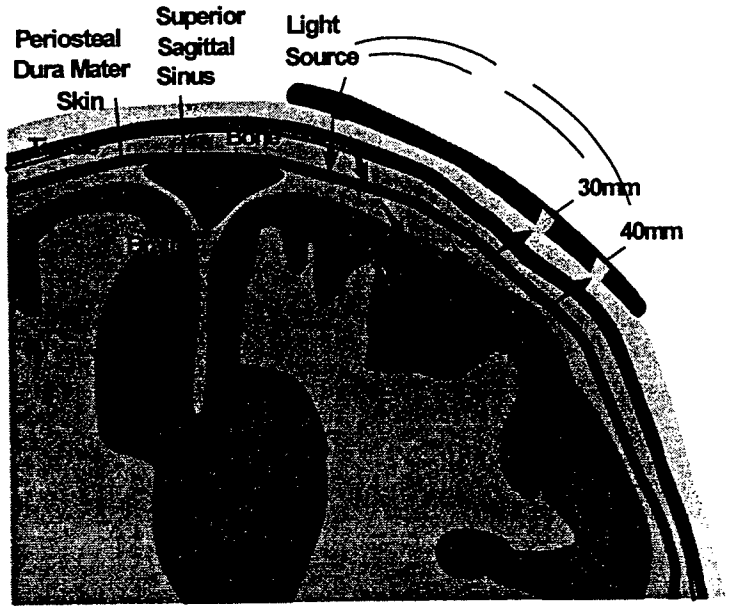
Eleven females with a mean (+/-Standard Deviation) age of 26 +/- 3 years, weight of 61.9 +/- 6.4kg and height of 164 +/- 7cm served as subjects. Each subject received medical clearance and gave their written voluntary consent to participate. Experimental procedures and protocols were approved by the Human Utilization Research Committee at Armstrong Laboratory, Wright-Patterson Air Force Base.

Subjects underwent tests for the assessment of cerebral hemodynamic responses to an orthostatic challenge. After skin degreasing and drying, the subjects were instrumented with a Somanetics Cerebral

Oxisensor which was connected to the Somanetics INVOS 3100 Cerebral Oximeter (Troy, Michigan). The oximeter was placed immediately below the hairline, and fully to the left of the midline (to prevent interference with reflectance due to bony midline structures) (10). All subjects had been transilluminated previously to rule out oversized left frontal sinuses, which could interfere with the Somanetics transmission of near infrared light causing questionable readings.

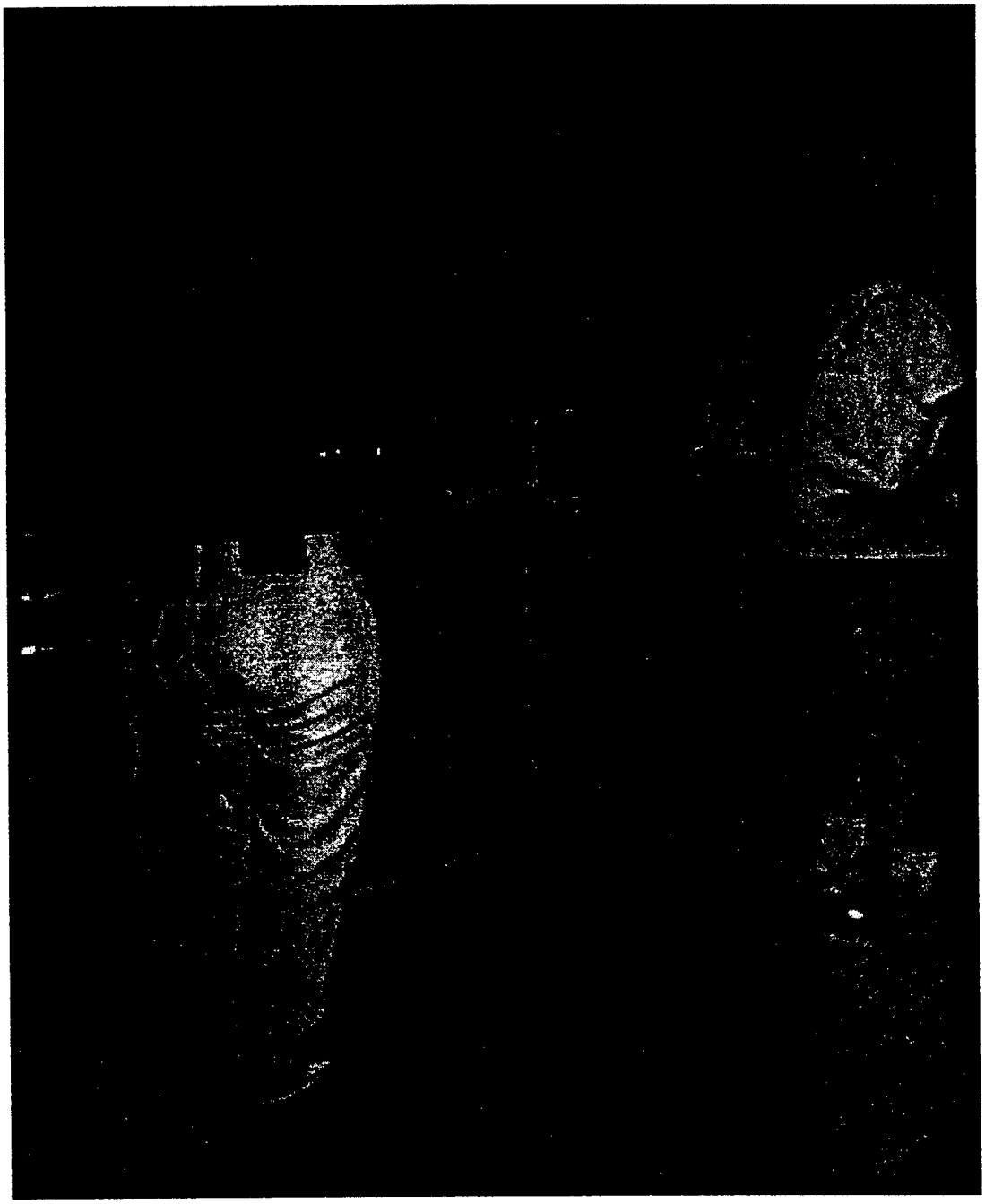
The Somanetic oximeter was developed on the basis that the typical adult head is too thick for direct transmittance through the brain. One alternative would be to penetrate a light path through the cranium. It is then reflected in all directions, which can be detected by an ipsilateral sensor. This alternative was used in the development of the Somanetics oxisensor. It emits a single, multi-wavelength light that essentially follows different arches to the two separate sensors. The near sensor receives light reflected from skin, subcutaneous tissue, and the cranium. The far sensor receives reflectance from those tissues as well as the first few centimeters of the brain. Based upon the Beer-Lambert law, an algorithm then uses the differentiated readings to effectively cancel out (or reduce) the influence of the shallower tissues (11).

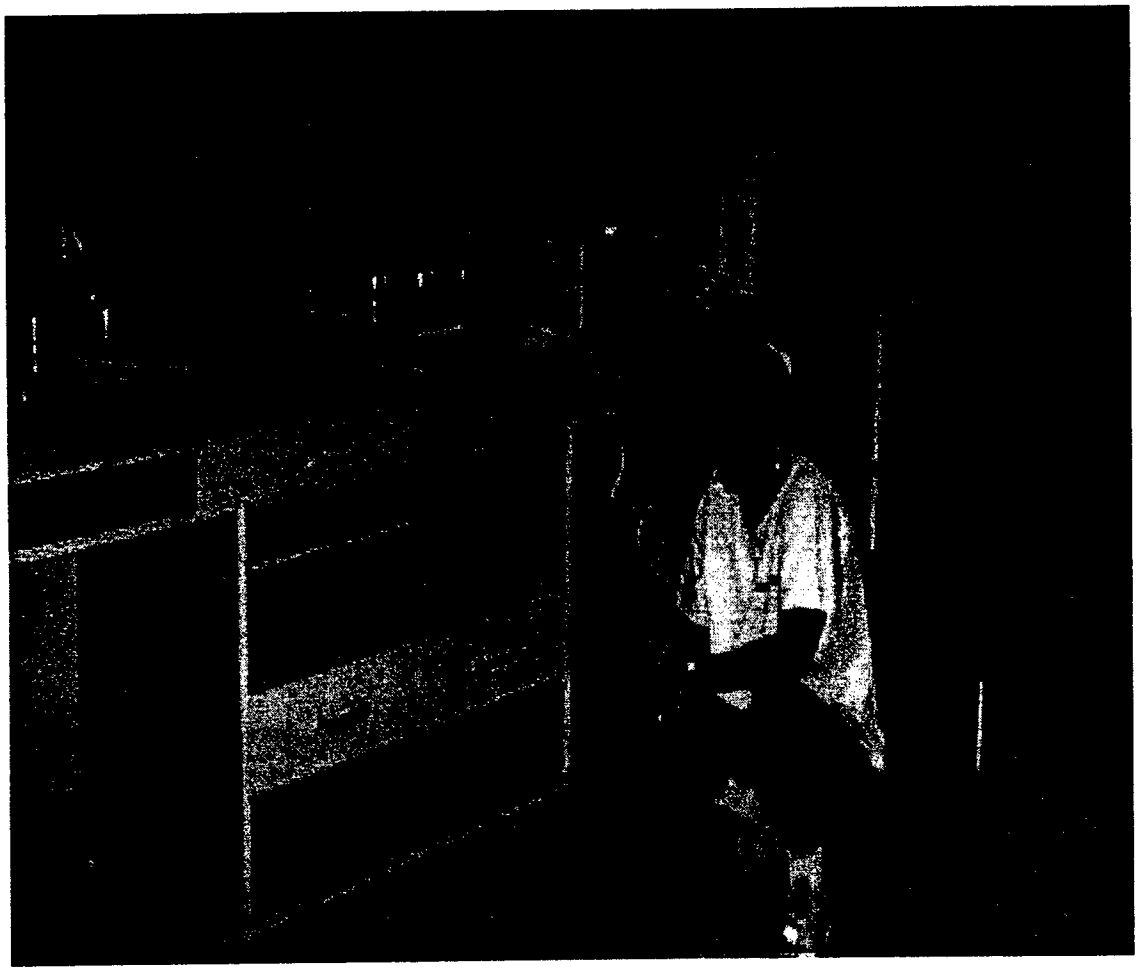
**Illustrates the Somanetics INVOS 3100 principles of operation.**




After instrumentation, subjects were instructed to assume a squatting position for a 4-minute time period. Regional cerebral oxygen saturation and relative cerebral blood volume data were collected at

approximately 5 second intervals throughout this test. The subjects were then instructed to stand erect and as quickly as possible without using their hands to assist them. Data were collected for 2 minutes after standing.





The relative cerebral blood volume values were derived from the following equation (provided by the Somanetics Corporation): 1-(X-Y)*100. The physiological data were stored on a Texas Instrument Travelmate 4000 WinDX microprocessor (Dallas, Texas). Data were evaluated by an univariate approach. More specifically, the student t-test was used to analyze data and the level of significance was established at 0.05.

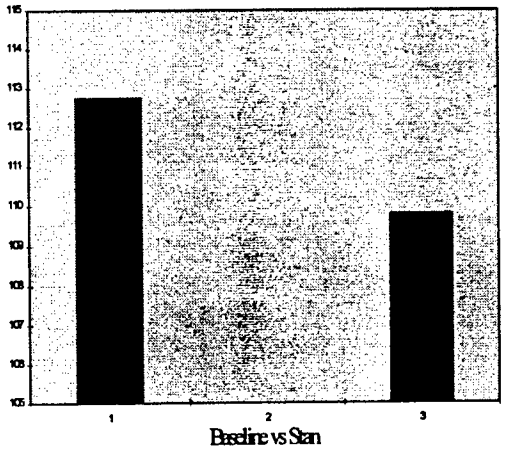
Results

The average baseline (squatting) relative cerebral blood volume for all ten subjects was 143.4 and the average blood volume proceeding the stand was 137.4. The average percent the cerebral blood volume changed from squatting to standing was -4.3%. The standard deviation for the average baseline, stand and the percent of blood volume dropped was +/- 25.4, +/- 25.8, and +/-2.8 respectively.

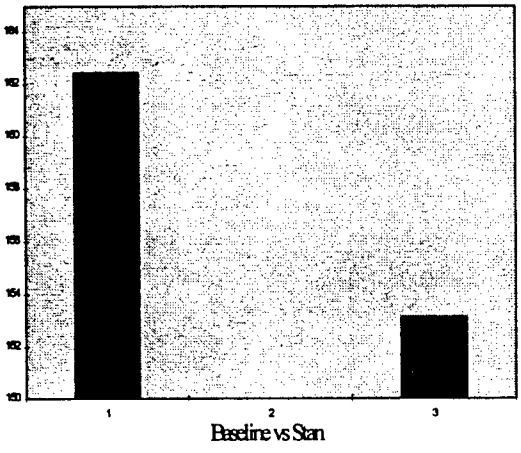
**Figure 1**

Individual Subjects Relative Cerebral Blood Volume

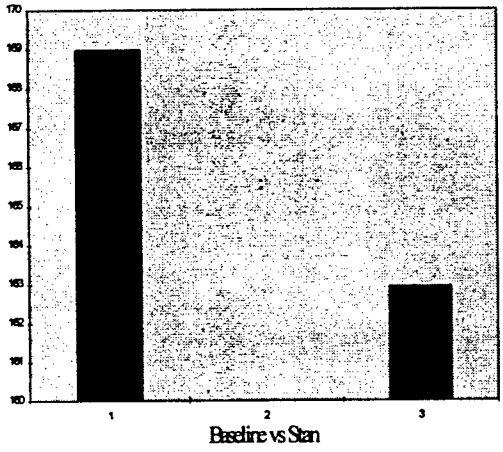
Subject #1 Squat-Stand Relative Cerebral Blood Vol




Subject #2 Squat-Stand Relative Cerebral Blood Vol




Subject #3 Squat-Stand Relative Cerebral Blood Vol

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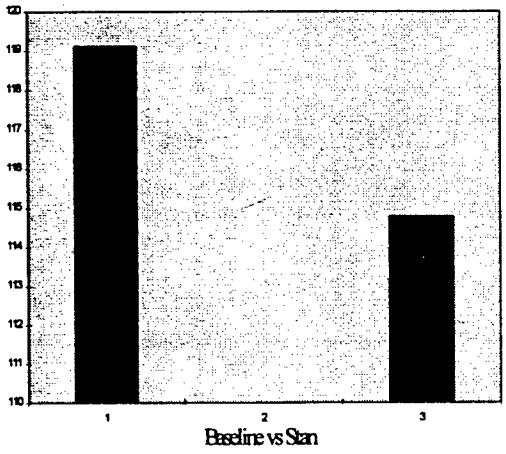


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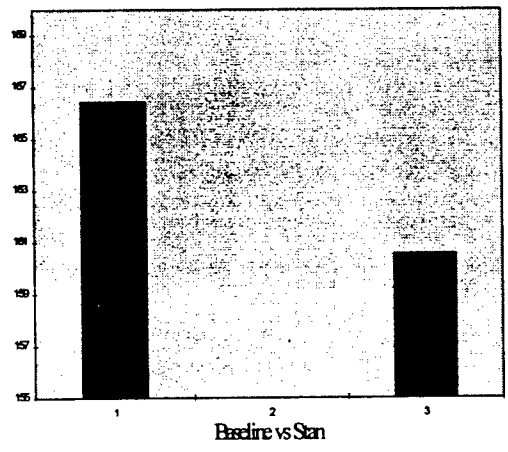


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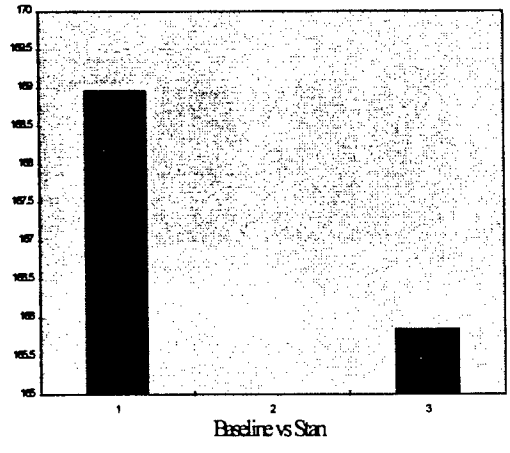


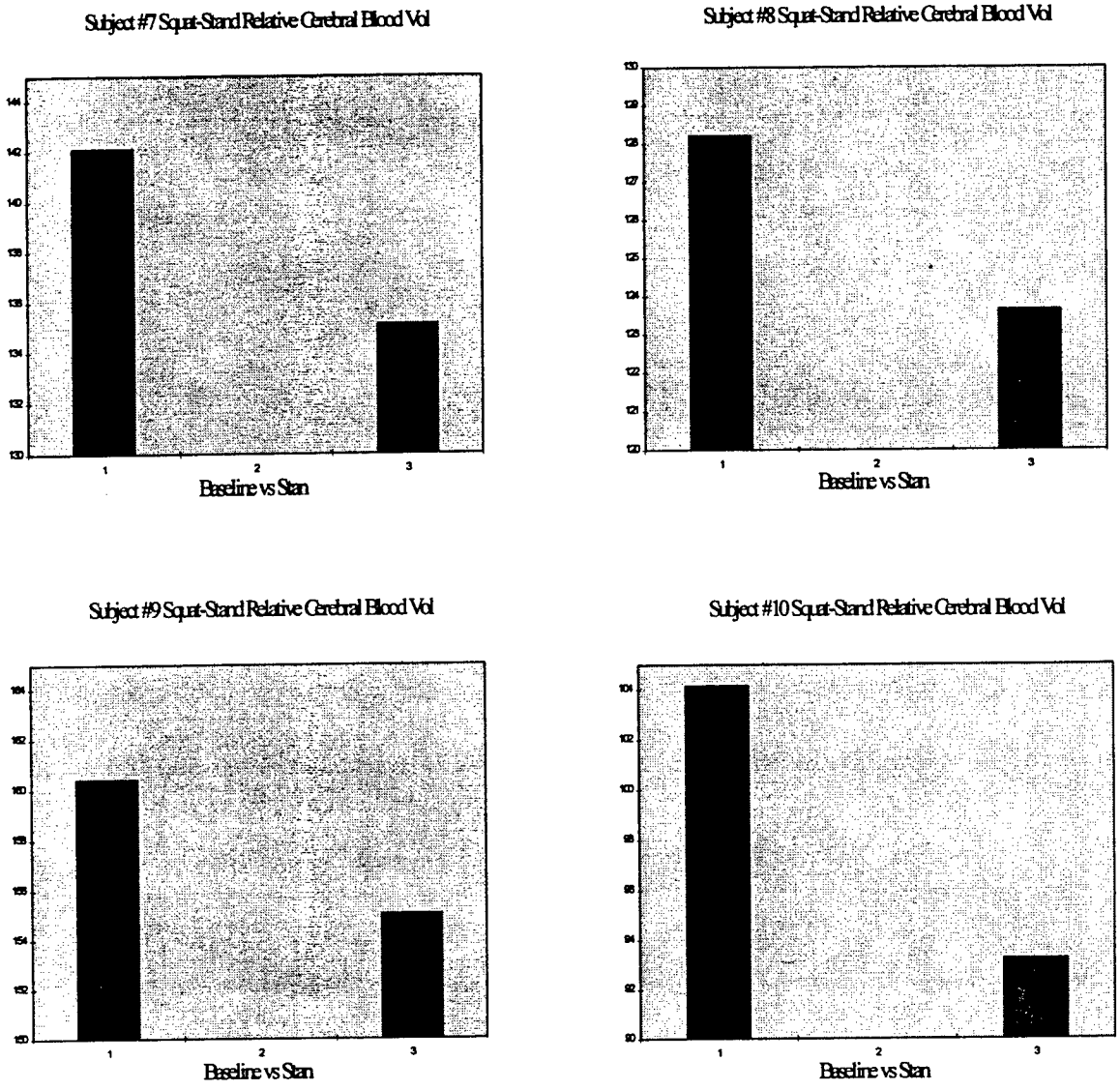

Subject #7 Squat-Stand Relative Cerebral Blood Vol
Baseline vs Stan
Subject #8 Squat-Stand Relative Cerebral Blood Vol
Baseline vs Stan
Subject #9 Squat-Stand Relative Cerebral Blood Vol
Baseline vs Stan
Subject #10 Squat-Stand Relative Cerebral Blood Vol
Baseline vs Stan

**Figure 2**

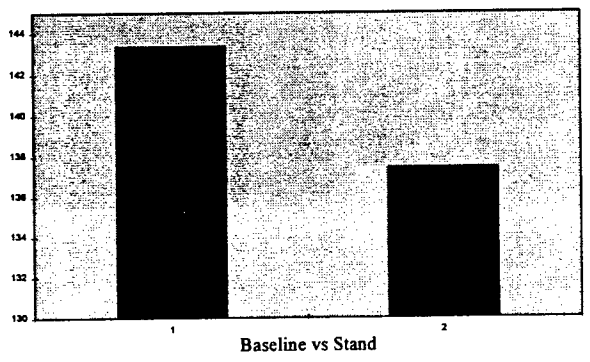

Average Squat-Stand Relative Cerebral Blood Vol (n=10)
Baseline vs Stand

**Table 1**

Relative Cerebral Blood Volume Data

| Subject | Baseline | Stand | % Drop |
|---|---|---|---|
| 1 | 112.8 | 109.8 | -2.7 |
| 2 | 162.5 | 153.2 | -6.1 |
| 3 | 169 | 162.9 | -3.7 |
| 4 | 119.1 | 114.8 | -3.7 |
| 5 | 166.5 | 160.6 | -3.7 |
| 6 | 169 | 165.9 | -1.9 |
| 7 | 142.1 | 135.1 | -5.2 |
| 8 | 128.2 | 123.7 | -3.6 |
| 9 | 160.4 | 155.1 | -3.4 |
| 10 | 104.2 | 93.3 | -11.7 |
| Average | 143.34 | 137.4 | -4.3 |
| St. Dev. | 25.4 | 25.8 | 2.8 |

Postural changes did not produce statistically significant changes in cerebral blood volume $p \leq 0.30$. These findings do not support the hypothesis that a blood shift foot-ward would result in a significant decrease in cephalad blood volume.

**Table 2**

Statistical Analysis Results

| *t-Test: Two-Sample Assuming Equal Variances* | *Variable 1* | *Variable 2* |
|---|---|---|
| Mean | 143.38 | 137.44 |
| Variance | 643.8173333 | 663.7737778 |
| Observations | 10 | 10 |
| Pooled Variance | 653.7955556 | |
| Hypothesized Mean Difference | 0 | |
| df | 18 | |
| t Stat | 0.519458031 | |
| P(T<=t) one-tail | 0.304885152 | |
| t Critical one-tail | 1.734063062 | |

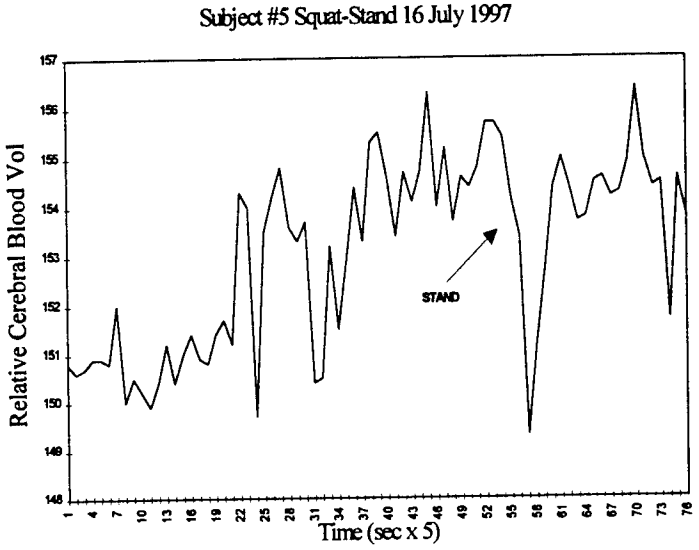
Discussion

This study measured the cerebral hemodynamic responses a subject would experience when performing an orthostatic challenge, the squat-stand test. The major finding of this study was that the cerebral hemodynamic response to the squat-stand test did not produce a significant amount of cerebral blood volume changes.

Physiologically, a change in posture from squatting to standing simulated greater gravitational

forces creating a hydrostatic pressure gradient within the cardiovascular system. This hydrostatic gradient causes the blood to redistribute into the lower extremities(12). The pooling of blood results in a cascade of events that include a reduction in the central venous pressure which produces an increase in relative cerebral blood volume(13). Figure 3 demonstrates how as time elapses and squatting continues, an increase in relative cerebral blood volume occurs.

**Figure 3**




Due to an immediate increase of acceleration forces in the +Gz direction and foot ward shift of blood upon standing, there is a sudden drop in cerebral blood volume. This is the body's physiological response to preserve basic function, such as circulation and respiration (14). Cerebral perfusion must be optimized in critical areas of the central nervous system, such as areas controlling brain stem reflexes. Falling cerebral blood volume triggers the brain to provide optional shunting blood away from non life-threatening areas of metabolism to those involved with reflexes established to ensure the integrity of the body. By reducing blood flow through the cerebral cortex, but not through the brain stem, the body is able to optimize reduced blood volume to the brain stem to sustain life until the body can adapt (12).

The results of this study may suggest that while females do experience all the physiological effects expected when they perform an orthostatic tolerance test, their bodies can adapt rapidly enough to the change in body position that the fluctuation in cerebral blood volume is insignificant.

Conclusions

This study establishes a foundation for experimentation involving the effect of +Gz on the relative cerebral blood volume. While no significant changes in cerebral blood volume were found when the subjects performed the squat-stand test under 1Gz, the response under various +Gz is unknown. This suggests further exploration for the effects of acceleration on the cardiovascular system. Because of the small number of subjects, limiting the study to one sex, and the characteristic similarities of the subjects (weight, height, and age) further experimentation is still necessary.

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# A STUDY OF PSYCHO-PHSIOLOGICAL EFFECTS ON BRAINWAVE ACTIVITY DURING VARYING LEVELS OF ACTIVITY

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**Final Report for:**
**High School Apprentice Program**
**Armstrong Laboratory**

**Sponsored by:**
**Air Force Office of Scientific Research**
**Bolling Air Force Base, Washington, DC**

**And**

**Armstrong Laboratory**

**August 1997**

# A Study of Psycho-Physiological Effects on Brainwave Activity During Varying Levels of Activity

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## Abstract

Recent advancements in flight technologies and the developments of newer technologies for use in upcoming generations of fighter aircraft have spawned the need for a greater understanding of the human factor in airplane flight. Increasing amounts of information surround the jet fighter pilots of today, adding to the levels of stress they face while flying the aircraft. The purpose of this study is to determine which levels of stress trigger certain types of brainwave activity, and demonstrate two key techniques which are used by researchers to better understand and predict the nature of brainwaves.

# A Study of Psycho-Physiological Effects on Brainwave Activity During Varying Levels of Activity

Max P. Vilimpoc

## Introduction

In the years since the introduction of the airplane into modern warfare, pilots have had to contend with a greater and greater flow of information as each new generation of aircraft arrived. Though each plane provided experienced and non-experienced pilots with more detailed information, the level of pilot stress during flight increased with the advancing technology. During high stress periods involving much pilot interaction with his plane, such as take-off, landing, and intense maneuvering, a condition known as "overload" could potentially lead the pilot to injury, during combat or non-combat training operations.

This potential for injury led to the funding for the development of an adaptive solution by the Armstrong Laboratories AL/CFHP division. While still a work in progress, the project involves elements from computer science, biology, and electrical engineering.

## Methodology

Using advanced data collection devices, which attach to key pilot skull points, the researchers gather information about brainwave activity. The information is recorded in the form of 60Hz multiple-sample readings of electrochemical activity apparent from the surface of the brain. This is accomplished by using highly sensitive contact electrodes and special minicomputers which record the data to high capacity hard disk cartridges. Later, the data is retrieved for analysis on more powerful research computers. Several approaches to analysis of the recorded information are taken.

Using specially designed software, the researchers hope to isolate bands of brainwave activity that react most predictably to high, medium and low stress situations. Once the bands of highest predictability are isolated, the researchers hope to create a working model of psycho-physiological response around which newer flight systems and information delivery systems can be designed.

A second approach towards the interpretation of the gathered data comes in the form of neural network pattern analysis. A neural network is a specially programmed matrix of computer codes that act most similarly to human neurons. Each cell of the matrix acts upon the data that is given to it, and accuracy is

established by the comparison of results between real data and training data. With each iteration of training, so the theory goes, the neural networks are designed to become more and more accurate at detecting patterns in the data. Using specially developed networks, the brainwave information is analysed for an accurately recognizable pattern. The goal of this approach towards the analysis of brainwaves is to develop a neural network that is 100% accurate in regard to the prediction of pilot stress levels.

## Results

Results from the neural network approach towards the detection of high, medium, and low stress levels have been between 70-97% accurate between the training and implementation of the trained neural networks. A main source of problems with the implementation of neural networks in real time has been the availability of processors capable of handling the amount of calculation and data generated by the human brain. So far, only recorded data has been used in brainwave analysis. Logically, the next leap for the researchers is to be able to analyse the real-time brainwave patterns of a pilot under high, medium, and low workload conditions.

## Conclusions

The convergence of low-cost processor technology and advances in the analysis of brainwave activity could soon allow near-future aircraft designers to implement stress-level monitors for pilots in both the commercial and military sectors. The benefits of this technology would allow for the plane's onboard computers to relieve the pilot of non-vital tasks under high stress situations, allowing the pilot to focus on his or her main objectives. Other applications for the results of this research may allow for better air traffic control, by monitoring the stress level of controllers and distribution information throughout flight tower computers on major airports such as La Guardia and LAX. The implementation of neural networks in air traffic control could efficiently manage the individual workloads of each controller, allowing for a form of computerized stress management.

# The Effect of Hyperbaric Oxygenation on the Mitotic Division of Prostate Cancer Cells

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Final Report for:
High School Apprenticeship Program
Armstrong Laboratory

Sponsored by:
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Bolling Air Force Base, DC

and

Armstrong Laboratory

August 1997

# THE EFFECT OF HYPERBARIC OXYGENATION ON THE MITOTIC DIVISION OF PROSTATE CANCER CELLS

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Theodore Roosevelt High School

Abstract

Prostate cancer is the most commonly diagnosed malignancy behind skin cancer. It is also the second leading cause of cancer death in U.S. men. Prostate cancer in its advanced stages is currently untreatable, thus there is a critical need for an effective treatment. For the experiment, malignant PC-3 cells were treated with hyperbaric oxygenation. It was hypothesized that the hyperbaric oxygen exposure will slow the growth rate of the cancer cells by stalling mitotic division. Effects of various pressure treatments ranging from 6 ATM to 0.32 ATM were tested. Experimental results indicated that .32 ATM was the most effective.

# THE EFFECTS OF HYPERBARIC OXYGENATION ON THE MITOTIC DIVISION OF PROSTATE CANCER CELLS

Elizabeth Walker

## Introduction

Hyperbaric oxygen is the inclusion of 100 percent oxygen at two to three times the atmospheric pressure at sea level. The resulting effect can include arterial oxygen tension in excess of 2000 mm Hg and oxygen tension in tissue of almost 400 mm Hg. The benefits of such oxygen doses include positive biochemical, cellular, and physiologic effects. The pressure expressed for hyperbaric oxygen is in multiples of the atmospheric pressure at sea level, which is 1 atmosphere. At sea level, the blood oxygen concentration is .3 mL per deciliter. Under the administration of 100 percent oxygen at ambient pressure increases the amount of oxygen dissolved in the blood fivefold to 1.5 mL per deciliter.

## Methodology

*Plating Cells:*

Prostate cancer cells were grown in flasks in RPMI-1640 growth media. For experimentation, the PC-3 cells were plated at 10^6 cells per plate in petri dishes. The plating of the cells was accomplished by decanting the media in the flask and adding a 2 mL wash of Trypsin-EDTA. This wash was decanted and then an additional 2 mL of Trypsin-EDTA was added. The flask was then incubated for 5 minutes at 37 C. After incubation the flasks were agitated to dislodge cells from the flask wall and 3 mL of RPMI-1640 media was added. The 5 mL cell solution was then pipetted vigorously to prevent cell clumping. After performing cell counts, cells were diluted to be plated in petri dishes at 10^6 cells per plate in 10 mL of media. The plates were then allowed to incubate overnight to ensure the binding of cells to the culture dish.

*Dosing Cells:*

After overnight incubation, plates to be dosed were decanted and 2 mL fresh media was added immediately prior to hyperbaric oxygen (HBO) exposure. Plates were then placed in the HBO chamber on a level surface to ensure even distribution of media/oxygen. The chamber was flushed at 10 ft. below sea level for 5 minutes. The pressure was then raised to the desired level at a rate of 1 ft/sec. Once the desired pressure was reached, the dosing continued for 1.5 hours. After the treatment period, the pressure was lowered at a rate of 1ft/sec to bring plates back to surface pressure. Plates then received an additional 8 mL of media and were returned to the incubator.

*Fixation of Cells:*

Plates remained in incubation for 23.5 hours. They were then spiked with 25 uL of 40 mM BrdU solution. They were then allowed to incubate 30 minutes. The cells were then harvested, saving all solutions in a 15 mL centrifuge tube. The media in the plates was decanted and a 1 mL wash of Trypsin-EDTA was added. This wash was decanted and an additional 1 mL of Trypsin-EDTA was added. The plates were then incubated for 10 minutes at 37 C. After incubation the cell solution was pipetted 30 times to break up and clusters and was then added to the 15 mL centrifuge tube. The plates were then washed with 1 mL of media two times, adding the remaining solution to the centrifuge tubes after each wash. The centrifuge tubes were then spun in the centrifuge at 1 K for 10 minutes. The supernatant was decanted and the pellet was resuspended in 1 mL of 70% EtOH over a period of 5 seconds while vortexing at the lowest possible speed. The fixed cell solutions were immediately placed in 4 C refrigeration and remained there overnight.

*Staining of Cells:*

The refrigerated cell solutions were spun in the centrifuge at 1 K for 10 minutes. After decanting the supernatant, the pellet was resuspended in 1 mL of PBTB. Each cell solution was then split into 2 microcentrifuge tubes (mct) to be stained. One mct of fixed cells received both Anti-BrdU and PI staining. The solution was centrifuged at 4 K for 8 minutes. The supernatant was decanted and the pellet was

resuspended in 1 mL of 2N HCl and then was incubated at 37 C for 20 minutes. The solution was then spun at 4 K for 8 minutes, decanted, and resuspended in 1 mL of 0.1 M Sodium tetraborate. The solution was allowed to incubate at room temperature for 5 minutes, was then spun at 4 K for 8 minutes, decanted, and resuspended in l mL of PBTB. This solution was immediately spun down at the above speed and time. After decanting the supernatant, the pellet was suspended in 20 uL of anti-BrdU FITC conjugated antibody and incubated at 37 C for 30 minutes. After incubation, 1 mL PBTB was added to the mct. The cell solution was then spiked with PI solution (final concentration 50 ug/mL) and RNAase (final concentration 10 ug/mL) and incubated at 37 C for 30 minutes, covered in foil. Following incubation, the stained cells were placed in 4 C refrigeration for no less than 30 minutes. The second mct of cells suspended in 500 uL of PBTB were stained with only the PI and RNAase in the manner described above.

Results:

The results of our experiment are as follows:

| **Cell Type** | **Cell Count** | | | | **Total** |
|---|---|---|---|---|---|
| Control PC-3 | 386 | 210 | 178 | 159 | |
| | 200 | 259 | 239 | 176 | 1807 |
| .32 ATM | 83 | 66 | 118 | 96 | |
| | 80 | 172 | 150 | 90 | 855 |
| .75 ATM | 133 | 144 | 161 | 120 | |
| | 133 | 126 | 139 | 126 | 1082 |
| 1.5 ATM | 145 | 142 | 200 | 150 | |
| | 152 | 115 | 118 | 105 | 1127 |
| 3 ATM | 101 | 106 | 115 | 146 | |
| | 118 | 138 | 145 | 241 | 1110 |
| 6 ATM | 120 | 200 | 108 | 111 | |
| | 93 | 104 | 93 | 103 | 939 |

Conclusion:

Based on the data collected, it can be concluded that hyperbaric-oxygenation can be used as an effective treatment for prostate cancer because Treatment would be most successful in stalling mitotic division at pressures of 0.32 ATM and 6 ATM. At .32 ATM, the number of cells decreased by 52.7% while at 6 ATM, the number of cells decreased by 48%.

# CG and MOI Study of Human and Manikin Segments

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**Abstract**

Anthropomorphic manikins are rigorously tested in both automotive and ejection seat testing. To properly simulate a human, these human surrogates should have proper centers of gravity (CG) and moments of inertia (MOI). The measurement and comparison of the CG and MOI properties of manikin segments was studied. The CG was measured using a scale and moment table while the MOI was measured using a mass properties instrument. This instrument is an inverted pendulum that measures periods of oscillations which can be computed to find the MOI along the principal and secondary axis. Afterwards, these measurements can be compared to similar manikin or human segments. Since it is not possible to use living human segments, regression equations have been developed through previous testing of cadavers and stereophotometry.

**Background**

The application of anthropomorphic manikins ranges from common, automotive crash tests to ejection seat testing. Anything that could be harmful to a human can be tested without possible injury. As time has passed, these manikins have evolved into complex (yet simple compared to humans) devices. From their origins as sandbags to muti -thousand dollar human surrogates with instrumentation, we can more accurately see the effects of possible life threatening situations.

To properly simulate a human, a manikin is designed with: anthropometry, biofidelity, repeatability, reproducibility, durability, sensitivity, and simplicity. Since there are many different sizes of humans, there are different sizes of manikins. Typically, most manikins are designed to simulate a 5th , 50th, and 95th percentile human with the average weight and size of each.

The first total body manikins were built in 1949 by Sierra Engineering Co. on contract for the USAF. These manikins were called "Sierra Sam" with many "brothers and sisters" coming later. In 1960, the Grumman-Alderson Research Dummy (GARD) manikin was developed. These were outfitted with Grumman (now Grumman-Northrop) telemetry packages and offered a manikin with proper whole body center of gravity (CG). These manikins have set the standard and are still in use today. As the automotive industry began testing for safety, many new manikins were developed. The Very Important People (VIP) manikin was built in 1966 by Alderson Research Laboratory (ARL) and became the first standard automotive manikin. Following, GM developed their Hybrid II in 1975 to test safety belts and refined it in 1976 with their Hybrid III manikin. The Hybrid models are now the automotive testing standard and are still in use today.

In the early '80s, it was decided that a more biofidelic and measurable manikin was needed for ejection testing. In 1986, Systems Research Laboratory (SRL), under

contract for Crew Escape Systems Technology (CREST), built the Advanced Dynamic Anthropomorphic Manikin (ADAM). The ADAM is capable of 64 channels of data from accelerometers and potentiometers (POTS) positioned in movable joints and the spinal column. These sensors track movement and possible harmful loads on a human.

Many other specialty manikins have been developed for testing. One such is a Side Impact Dummy (SID). Since normal, full body manikins cannot accurately measure large side impacts, SIDs were developed. Another manikin in development is the Sea Water Immersion Manikin (SWIM) for testing floatation devices. Many other manikins that have not survived the rigorous use for which they were intended or were superseded by a better equipped replacement. Such manikins include: Dynamic Dan, Repeatable Pete, and Supermorphic.

**Method**

The application of many manikins include automotive and ejection seat testing. Present day ejection seats are designed with a CG envelope, a small football size pocket that the pilot's CG must fit into for the seat to perform as it should. With the loosening of pilot requirements, the Air Force is verifying if current ejection seats will accommodate this increased pilot population. Two new test manikins have been developed to test these outer limits of pilot weight and size.

First, the Joint Primary Aircraft Training System (JPATS) is used to simulate a 99th percentile male seat occupants. This 245 lb. "monster" of a manikin is now being measured to find if it truly represents a human its size and weight.

And second, the Lightest Occupant In Service (LOIS) is used to simulate a 5th percentile female. This "doll" is a 103 lb. manikin now being used in testing.

The center of gravity and moments of inertia for both the segments and the

whole structure of an anthropomorphic manikin was studied. The study was conducted in the CFBV (Vulnerability Assessment Branch, Armstrong Laboratory) lab using: an electronic scale, moment table, mass properties instrument, and an electronic digitizer. Each of these will be explained below.

An electronic scale is used to find the weight and CG of a composite. The CG is found by placing a moment table on the scale . The composite is placed on the table at its different axis which gives different weights ($F_{CG}$). Since the length of the moment table is known ($R_G$), and the true weight of the composite ($F_G$), the CG distance from one end of the table ($X_{CG}$) can be found using:

$$X_{CG} = F_G R_G / F_{CG}$$

These data are used to ensure the CG of the segment is about the measuring axis of the mass properties instrument.

A mass properties instrument measures the periods of oscillations of an inverted pendulum and platter. The placing of an object on this platter changes the oscillation characteristics which can be recorded and used to find the MOI of the segment and box composite.

To acquire quantitative data on the position of the CG, the composite is digitized according to either anatomical or mechanical landmarks. For the upper leg segment, such landmarks include: lateral and medial points on the knee axis, different points on the hip ball, and so forth. These landmarks are standard and have been determined through previous experimentation.
With the exact location of the CG known in relation to these set landmarks, it can be compared to other manikin segments or their human counterparts.

Since the weight, CG, and MOI of human segments cannot be measured with a live segment, many different methods of gathering these measurements have been

devised. First, cadaver studies were accomplished to gather this data. Due to the lack of certain fluids in a cadaver segment, these studies are limited. Second, regression equations were formulated through different studies. Such questions as "If the leg is this long and has this much volume" can fairly accurately predict a segment weight, CG, and MOI. And last, whole body laser scans are performed on whole body and human segments.

Measurements of the large male JPATS manikin segments were performed during the weeks of 13 July through 27 July. These measurements included: CG, MOI, and weight calculations. This data can be compared to predicted human measurements through regression equations from former studies. The CG is stated as a percentage of the position down the segment from anatomical landmarks. Following is only one set of data collected and compared, but gives a good representation of what is considered in a test.

**Results**

| | Segment | Weight(lbs.) | Center of Gravity (%) | Moments of Inertia(gm/cm^2) | | |
|---|---|---|---|---|---|---|
| | | | | X | Y | Z |
| Manikin | right upper leg | 26.20 | 46.23 | 3304060 | 3304060 | 318001 |
| Human | right upper leg | 30.22 | 45.23 | 2418011 | 2562784 | 735952 |

**Discussion**

The predicted and measured CG and MOIs did not come out very close. There are a few reasons this is true. First, almost all the weight of a manikin segment is in the "bone", unlike a human segment where the weight is primarily in the surrounding muscle tissue. Experimental "bones" have been made out of titanium and carbon fiber to take weight out of the structure and redistribute it near the "flesh". This would increase the MOIs, especially about the Z-axis. Second, the brass knee was replaced with a lighter, aluminum knee. During early testing, the CG of the leg was more than one inch below

where it should be. By replacing the knee, the CG shifted over one inch away from the knee, to make it more correct. And third, future studies will have to be made to shift weight inside the manikin and to correct the MOIs.

**Conclusion**

Of all the things that one would want to save during a plane crash is the pilot. The pilot is the most expensive single object on the plane. The ejection seat is the means to shelter the occupant out of the plane. It can be honestly stated that the ejection seat and parachute is the most important object during an ejection.

The goal of an ejection seat is to safely eject the pilot out of the plane, clear the plane, stay stable in the air, slow down to speeds capable of having a parachute deployed, and then let the occupant float to the ground. If one of these things is not accomplished, it is not likely the pilot will survive.

It was stated earlier that the CG of the manikin must be correct for ejection seat testing. That is only partially true. It is true that the occupant and seat composite must be CG correct for testing. Sometimes ballasting can be used to make the composite CG correct. It is not practical to give each pilot a ballasting pack for every flight, so the CG of the pilot must be correctly placed in relation to the seat. If the composite CG is not correct, the seat characteristics change causing a possible pitch, yaw, and or roll producing an unsafe ejection. The goal is to give the pilot the safest ejection possible. It would not be moral or practical to continually lose pilots.

Through continual modifications and testing of manikins, a more representative surrogate can be constructed to help with ejection testing. There is not only concern with the CG and MOI of a manikin, but with the motions and anthropometry of our hard headed friends. Even with this testing, a one hundred percent representation of a human will never be found due to the complexity and detail of the human body.

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# DETECTION OF CLOSTRIDIUM DIFFICILE TOXINS BY POLYMERASE CHAIN REACTION

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# DETECTION OF *CLOSTRIDIUM DIFFICILE* TOXINS BY POLYMERASE CHAIN REACTION

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## *Abstract*

*Clostridium difficile* is an anaerobic prokaryote. It is a major cause of antibiotic-associated diarrhea and pseudomembranous colitis. *C. difficile* is one of the most commonly detected enteric pathogens and an important cause of nosocomial infections in hospitals and nursing homes. In most severe cases *Clostridium difficile* induced colitis can lead to death. The standard methods of diagnosing *Clostridium difficile* induced colitis are time consuming and lack specificity. Scientists and clinicians have called for polymerase chain reaction-based assay to detect gene encoding toxins A and B in stool samples.

## *Introduction*

*Clostridium difficle*-induced colitis (CDIC) is caused when toxigenic forms of *C. difficle* overgrow the gastrointestinal tract. *C. difficile* is found in about 5% of all adults and usually does not cause problems. When problems do occur, it is generally due to overgrowth caused by three predisposing factors (Reinke and Messick, 1994): the presence of toxigenic strains, the disturbance of intestinal microflora, and the presence of risk factors (i.e. advanced age, illness, prolonged hospitalization...).

The standard methods used for diagnosing CDIC is an assay for cytotoxin B which takes 24-48 hours. This is usually done concurrent with the culture of the organism from stools, a process that takes about 48 hours (Reinke and Messick, 1994) . Other methods used are enzyme immuno assay tests and latex tests which take a short amount of time but lack sensitivity and precision.

A single, rapid, sensitive and specific test to determine the presence of toxigenic forms of *C. difficile* in stool samples is needed. Therefore, scientists have called for and have begun using a polymerase chain-reaction assay to detect the genes encoding toxins A and B in stool samples (Gerding, 1996). Also in use is a procedure to extract *C. difficile* DNA directly from stools of a quality sufficiently pure to be used for PCR procedures (Arzese et al., 1995).

## *Materials and Methods*

### Bacterial Strains and Culture

Clinical isolates were obtained from the Bacteriology Section, Epidemiologic Research Division, Armstrong Laboratory, Brooks Air Force Base. Bacteria were routinely growing in blood agar.

### NUCLEIC ACID MANIPULATIONS:

A single colony (ca. 1 mm diameter) was isolated from an agar plate and mixed with 200 µl of sterile distilled $H_2O$ in a microcentrifuge tube by vortexing briefly. The resuspended colony was boiled for 17 minutes. Amplification was carried out in a lreaction volume of 50µl, containing (for a 1x reaction) 2 µl of chromosomal DNA, 32.575 µl of $dH_2O$, 5 µl of buffer 1 (pH 8.3, 100 mM Tris-HCl, 15 mM $MgCl_2$, 250mm KCl), 0.5 µl Taq, 3.125 µl dNTP mix, and olgionucleotide primers specific for each fragment to be amplified. The primers used for PCR were ToxA-P1 (5' GGAAATTTAGCTGCAGCATCTGAC 3'), ToxA-P2 (5' TCTAGCAAATTCGCTTGTGTTGAA 3'), ToxB-P1(5'GGTGATATGGAGGCATCACCACTAG 3'), and ToxB-P2(5' TCCAGGATAAGTCTCCTCTACGTTG 3'). The optimum primer concentration was 0.02 µM.

Amplification was conducted for 37 cycles, using a Perkin-Elymer 9600 thermocycler. Each cycle consisted of 5 minutes at 94°C, 1 minute denaturation at 94°C, 1 minute annealing at 41°C, and 1 minute extension at 72°C. At the conclusion of the PCR cycle the tubes were incubated for 7 minutes at 72°C to allow the amplification process to come to completion.

The PCR amplified gene fragment was run on a 1% agarose gel for 1 hour, stained with ethidium bromide and photographed. See Fig 1 for results.

## *Results*

The electrophoresis results for Fig.1 were inconclusive. The primers can be seen but the expected bands were not present. We were looking for bands around 1-2kbp in lanes two and four. As our positive control we used a sample (#5989) already demonstrated to work, and as our negative control we used distilled $H_2O$. Lanes one and six contained 4 μl of λ Hindi III ladder, lane two had the positive control with undiluted tox A and B primers, lane three had a negative control with undiluted tox A and B primers, lane four contained a positive control with undiluted tox A and B primers, and lane five had a negative control with diluted tox A and B primers. When dilution was utilized the primer was diluted 1:10. The λ Hindi III marker DNA ran as expected, and the 500 bp, 2.0, 2.2, 6.6, and 9.4 bands were clearly visible.

## Discussion

Prior to the final electrophoresis gel run (*Clostridium difficile*) there were many attempts to produce reliable data by varying conditions (e.g. Toxin primers, $MgCl_2$, DNA concentration]. We took into account the possible errors, some of which included: not measuring the quantities correctly, pipettes not properly calibrated, use of the wrong buffer, the PCR thermocycler incorrectly programmed, incorrect estimation of amount of master mix, or even using too much DNA. All of these possible problems were thought to have been resolved in the final electrophoresis run. There was consultation with Sharon Rose, who performed this experiment successfully using the same master mix. We concluded that the main problem was due to too much pure DNA extract. With this problem resolved the final run was performed. These results still proved to be inconclusive. The only feasible explanation for such inconclusive data is human error.

Since this was previously performed successfully I feel with more trials conclusive data can be achieved and this would be an effective method. It has also been suggested (Dr. Joe Shaw, Auburn University) to use a GeneComb™ amplicon detection method in conjunction with the multiplex PCR and DNA extraction procedure. This can successfully reduce the assay time, vital in a clinical setting. I performed a trial GeneComb™ run with *E. coli.* to perfect technique and attain knowledge on the assay (see Appendix II.)

As well as working with *Clostridium difficile* and the GeneComb™ I also experimented with *Candida parapsilosis* (see Appendix I)

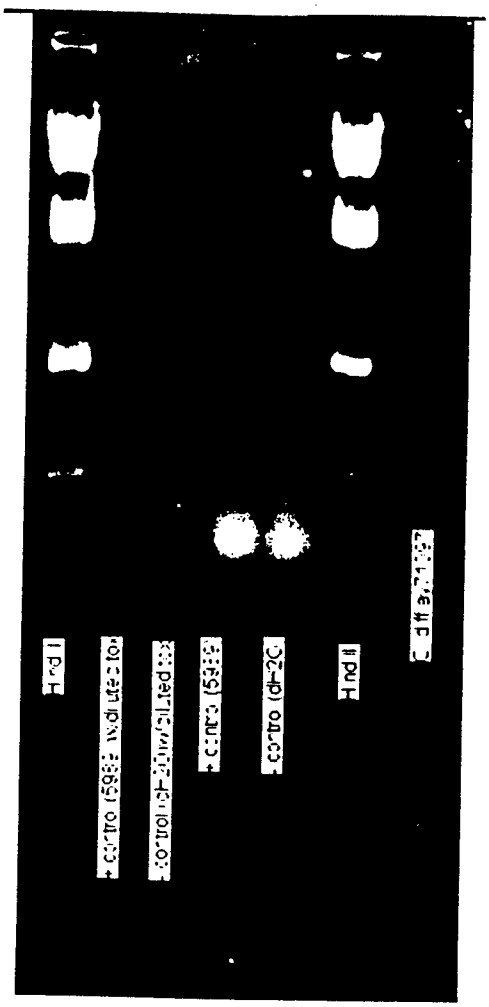
My eight week stay has proven to be quite productive. I gained much knowledge on the basic laboratory and the basic methods of scientific research. I learned basic laboratory techniques and procedures, and became acquainted with laboratory tools, and basic lab safety. Along with acquiring knowledge about the lab I also gained hands-on experience in the isolation of DNA and the performing of

Polymerase Chain Reactions. Not only did I have the opportunity to work in the lab, I also learned how to edit scientific research papers and the proper method in documenting references.

Many of the things that I had access to during my short stay here I would not have ever been able to experience if not for the High School Apprenticeship Program and the people at Brooks Air Force Base Armstrong Laboratories. This apprenticeship has allowed me to work in a "true" lab setting which has allowed me to experience the "ins-and-outs" of scientific research.

***Figure 1***

*Clostridium difficile*



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# Appendix One: *Candida parpasilosis* PCR

## *Materials and Methods:*

### Bacterial Strains and Culture (see Fig. 2)

Samples were from Dr. Mike Rinalde at Mycology Reference Laboratory, Audi Murphy VA Hospital, San Antonio, Texas (see Fig. 2).

### NUCLEIC ACID MANIPULATIONS:

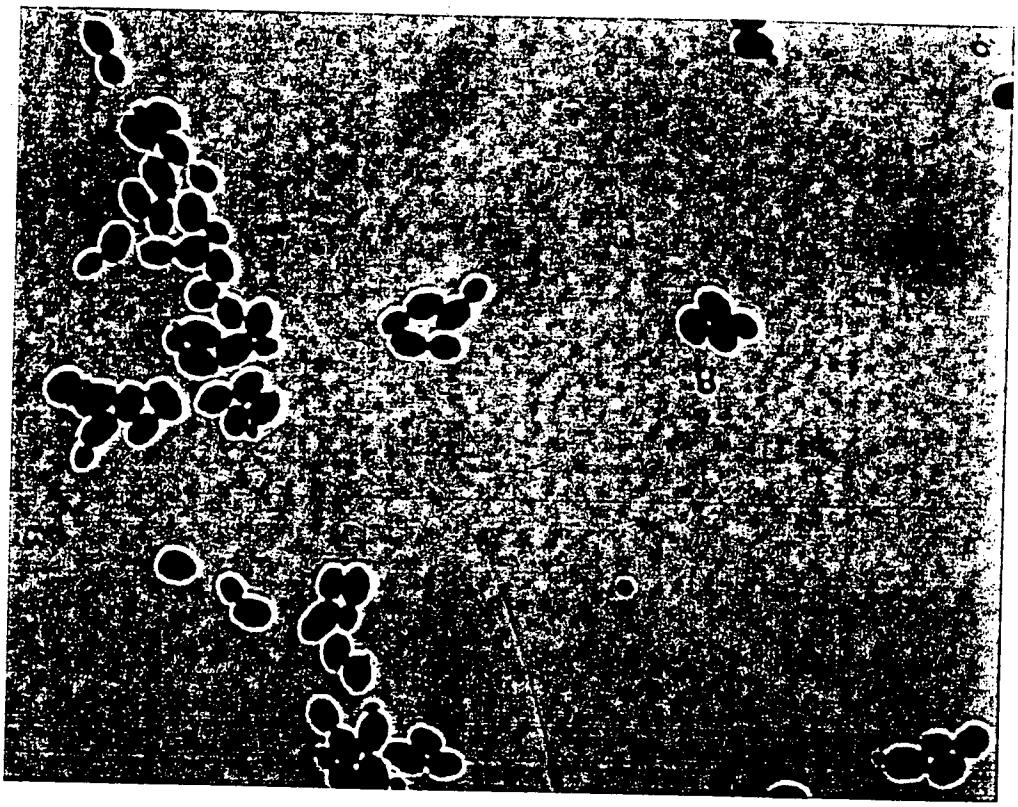
All steps were carried out under the hood. A single colony was isolated from an agar plate (made up of 450 ml of Yeast Peptone Dextrose broth and 2% agar) and inoculated into a 50 ml YPD broth. For DNA isolation, 1.5 ml of the broth was pipetted out and spun at 3000 rpm for 2 minutes (1.0 ml was pipetted out to prepare frozen glycerol stock), then spun in 1 ml of 1 M of sorbital, and resuspended in a 1 ml mix of 4 ml of 1 M sorbital 50 mM Potassium Phosphate pH 7.5, 4 µl B-Mercaptoethanol, and 30 µl of lysozyme. The mixture was incubated for 30 minutes at 30°C shaking slightly at 40 rpm.. After the incubation period the sample was pelleted again to pack the cells (3000 rpm for 2 minutes) After removing the supernatant, pellets were resuspended in 250 µl EDTA, 50 µl of 10% SDS and 2.2 ml of $dH_2O$ (each tube received 500 µl). For lysis 3µl, of Protinese K was added and the tubes were placed into a dry bath for 3 hours at 50°C inverting every 10 minutes. After the dry bath the DNA was purified three times with phenol chloroform using phase lock gels. After the purification, 2 vols. of 100% ETOH was added, mixing by inversion. When DNA becomes visible it was spun for 5 minutes at top speed. The next steps can be performed on the bench. The pellets are air dried for 5 minutes, then dissolved in 100 µl of TE and stored at 37°C oscillating. After the pellets were fully dissolved 3 µl of RNAse wase added and then the samples were placed in 37°C again for 30 minutes (not oscillating). After 30 minutes, 4 vols. of isoproponal was added to each tube then inverted and centrifuged for 3 minutes at top speed. Following centrifuging the samples were washed in 70% ETOH (1 ml) with slight shaking, and then spun again for 3 min. The supernatant was then disposed of and the samples were allowed to air dry completely. The DNA was then dissolved in sterile distilled $H_20$ (100 µl) and placed in a 50°C bath for one hour, and refrigerated at 4°C over night.

To check the concentration of DNA in the samples a spectrophotometer was used. See Fig. 3 for results.

We checked the quality of the DNA on a 0.7% agarose gel for one hour, stained with ethidium bromide and photographed. See Fig. 4 results.

***Figure 2***

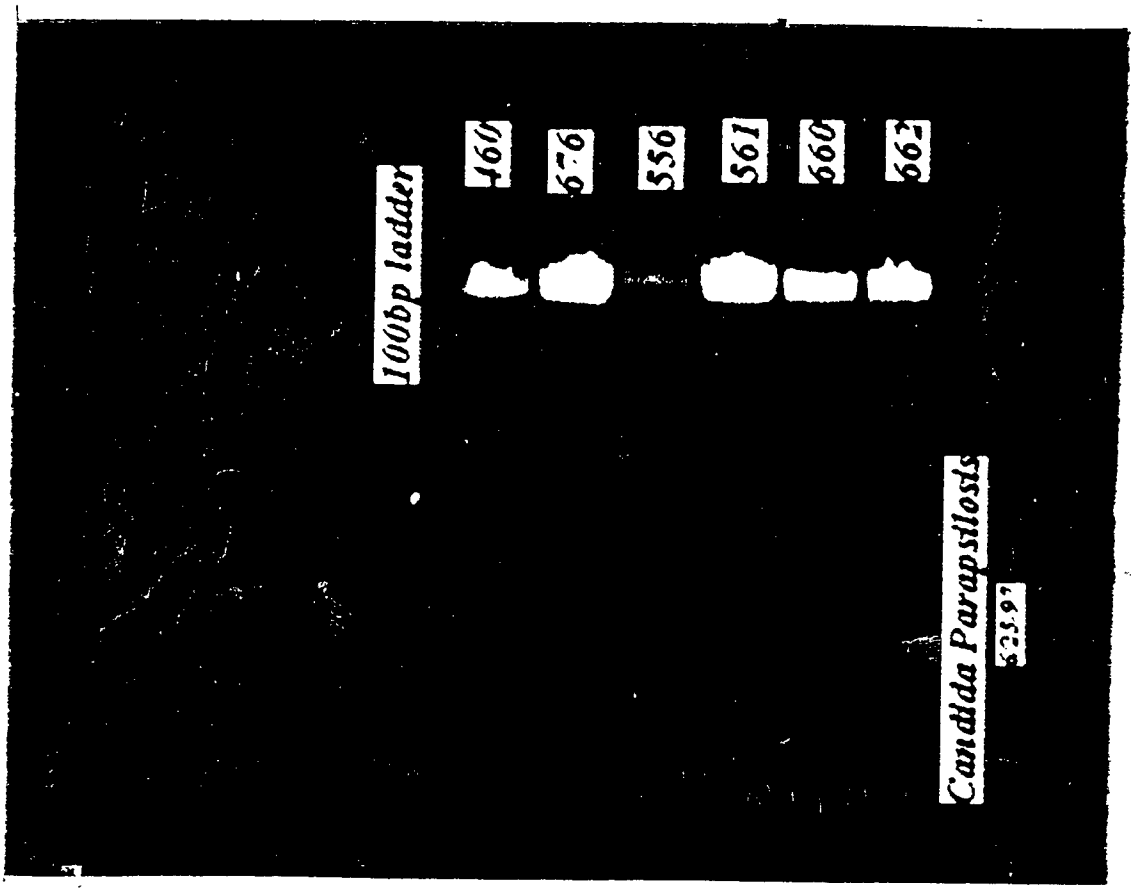
*Candida parapsilosis*



*Figures 3 and 4*

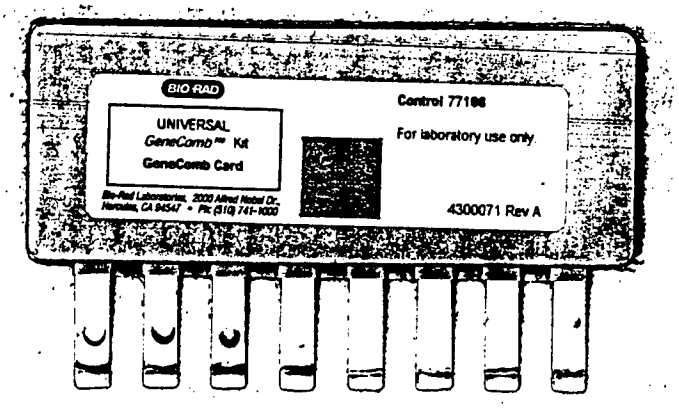
**Spectrophotometer readings for *Candida parapsilosis***

| SAMPLE | 260 | 280 | 320 | RATIO | CONCT. ng/µl | +dH2O µl |
|---|---|---|---|---|---|---|
| 460 | 2.275 | 1.196 | 0.208 | 2.092 | 103.35 | 0 |
| 676 | 2.777 | 1.952 | 0.176 | 1.465 | 129.90 | 0 |
| 556 | 0.786 | 0.425 | 0.154 | 2.33 | 30.60 | 0 |
| 561 | 2.695 | 1.721 | 0.229 | 1.653 | 123.30 | 50 |
| 660 | 2.686 | 1.714 | 0.248 | 1.663 | 121.90 | 100 |
| 662 | 2.567 | 1.505 | 0.215 | 1.822 | 117.55 | 0 |



# Appendix two: GeneComb™

*Figure 5*



# Detection of PCR* Amplicons Diagnostic of *E. coli* O157:H7 with the Universal GeneComb™ Kit

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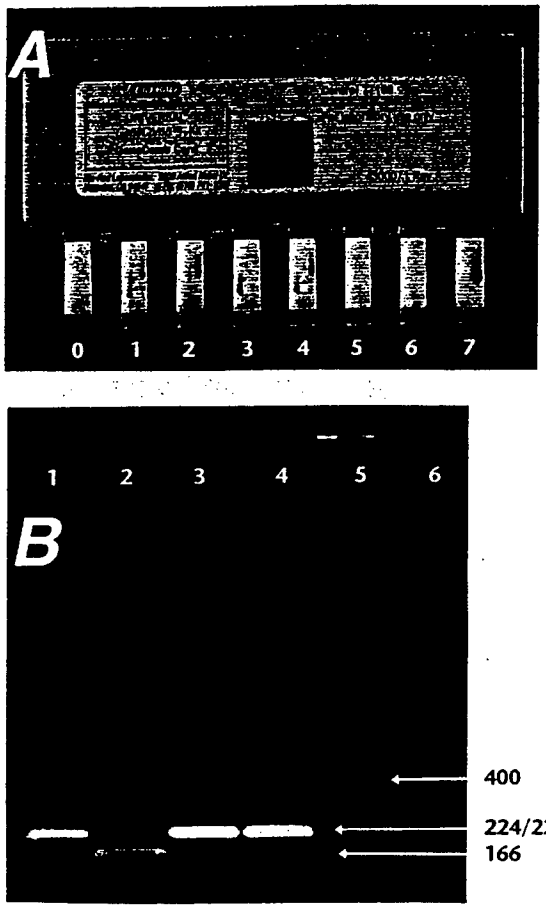


**Fig. 1. Comparison of GeneComb and electrophoresis results.** A. GeneComb, upper position of each tooth loaded with probe specific for the 166 bp amplicon that is diagnostic of *E. coli* O157:H7 (1). Lower position of each tooth loaded with the two probes specific for the two Shigella-like toxins of *E. coli* O157:H7 (2). Lane 0, negative control (water only); Lane 1, amplicons from strain A45; Lane 2, PCR amplicons from strain A57 (with neither toxin gene); Lane 3, amplicons from strain A59 (cured of 60 Mda plasmid); Lane 4, amplicons from strain A21; Lane 5, amplicons from *E. coli* DH1 (A48); Lane 6, *E. coli* 0126 (A130); Lane 7, internal standard provided with GeneComb kit. B. Electrophoresis results. Lanes are the same as in Fig. 1A (above), but lane 0 and 7 are not shown.

## Introduction

*Escherichia coli* O157:H7 is an important pathogen which causes hemorrhagic colitis and hemolytic uremia syndrome (HUS).[4,6] Disease outbreaks due to *E. coli* O157:H7 have been reported in nursing homes[5] and day care centers.[6] *E. coli* O157:H7 isolates typically possess a 90–100 kb plasmid that encodes pathogenic functions, and also express two distinct phage-encoded *Shigella*-like toxins (SLT I and SLT II). Various authors have demonstrated the diagnostic value of probes specific for these molecules.[2,3]

One prime consideration for the routine application of PCR-based detection methods for detection of *E. coli* O157:H7 is the ability of personnel to easily carry out the various steps. These steps can be conceptually divided into three phases: 1) sample preparation, 2) amplification and 3) detection of the amplicon. Detection of the amplified DNA, is usually accomplished by agarose gel electrophoresis and subsequent viewing of the ethidium bromide-stained PCR product. This step has remained largely unchanged since the advent of PCR, even though many advances have been made in the first two phases. In our experience, clinically trained personnel can often experience difficulty in preparing and casting the gel, loading the samples in the gel, performing the actual electrophoresis, staining the gel, visualizing and photographing the product, and interpreting the result.

We have examined use of a chromatography-based method (Universal GeneComb kit, Bio-Rad, Hercules CA) to detect PCR amplicons specific for either: 1) the plasmid of E. coli O157:H7, 2) the *Shigella*-like toxin genes of *E. coli* O157:H7 (SLT I and SLT II). The goal was to determine the efficacy of chromatography-based methods to detect biotinylated PCR amplicons as well as their potential usefulness in situations where laboratory personnel might have limited access to equipment and facilities. A total of 40 bacterial strains were examined, including 20 *E. coli* O157:H7 strains and 20 other *E. coli* strains or *Shigella* spp(Table 1).

# Materials and Methods

## Nucleic acid manipulations

Genomic DNA was extracted from *Escherichia* and *Shigella* strains for PCR as described by Fratamico *et al.*[2] Briefly, a single colony from an overnight culture was resuspended in 200 µl of lysis buffer (0.5% Triton X-100, 20 mM Tris pH 8.0, 2 mM EDTA) and boiled for 10 minutes to lyse the cells. All oligonucleotides were prepared by Midland Certified Reagent Co. (Midland TX). Where required, oligonucleotides were biotinylated by addition of a 5' biotin-labeled T residue.

Two primers (MK1 and MK2) were employed to amplify the SLT sequences that are diagnostic of O157:H7 isolates. The sequence of MK1 is 5'TTTACGATAGACTTCTCGAC3' and that of MK2 is 5'CACATATAAATTATTTCGCTC3'. Two different oligonucleotide probes based upon the report of Karch and Meyer[3] were employed to probe for each of the two different SLT amplicons. The oligonucleotide, MKP1, 5'GATAGTG-GCTCAGGGGATAA3', was synthesized to detect SLT I. To detect SLT II sequences the probe MKP2, 5'AACCACACC-CACGGCAGTTA3' was constructed.

Primers, MS1F and MS1R, 5'ACGATGTGGTTTATTCTGGA3' and 5'CTTCAGTCACCATACATAT3', respectively, were used for specific amplification of a 166 bp fragment of the 60 Mda plasmid that is found in *E. coli* O157:H7 strains.[2] The oligonucleotide probe, MSFPP, 5'CCGTATCTTATAATAAGACG 3', was prepared to detect the 166 bp plasmid amplicon.

Five to 10 µl of colony lysate along with 50 pmol of each primer was added to an amplification volume of 100 µl. A multiplex PCR protocol[2] was employed to amplify the plasmid sequence and both SLT sequences. The reaction mix was 2.0 $MgCl_2$, 20 mM Tris (pH 8.0), 50 mM KCl, 0.001% gelatin, 200 µM for each of the four dNTPs (800 µM total), with amplification as follows. An initial denaturation of 94 °C for 5 minutes was followed by 35 cycles of denaturation (1 min, 94 °C), annealing (3 min, 48 °C) and extension (4 min, 72 °C), followed by a single period of extension for 5 minutes at 72 °C.

Following amplification, 10 µl of the PCR reaction was analyzed by agarose (1.6%) gel electrophoresis and subsequent visualization with ethidium bromide. Additionally, another 10 µl were analyzed by means of the Universal GeneComb kit (Bio-Rad, Hercules CA). The GeneComb card has eight nitrocellulose teeth, one of which is reserved for a control reaction. On each tooth, one or two probe spots may be utilized for evaluation of the PCR products. In all of our tests, two probe spots per tooth were utilized. Ten pmoles of oligonucleotide probe were deposited onto the teeth in accordance with the manufacturer's instructions. The probe was covalently affixed to the nitrocellulose by a three minute exposure on the same ultraviolet transilluminator that was utilized for visualization of ethidium bromide stained amplicons. Chromatography of the amplicons and color development of the duplex molecules were as described by the manufacturer. A purple spot on the comb, in the area of the probe was recorded as a positive result, the lack of a spot in the expected area was recorded as a negative result.

To examine the flexibility of the comb, a simple experiment was designed to determine if a single probe spot could provide information about multiple amplicons. Thus, in some determinations, the probe for SLT I (MKP1) and the probe for SLT II (MKP2) were mixed together in equal portions and both were added to a single probe spot on a tooth.

# Results

## Electrophoresis results

Figure 1B shows typical electrophoresis data for the PCR reactions. Two bands of the expected sizes (166 bp and 224/227 bp) were produced from the DNA isolated from the O157:H7 strains. There were two exceptions. One O157:H7 strain obtained from the American Type Culture Collection does not produce either SLT I or SLT II (A57), thus as expected, only a 166 bp amplicon was obtained from this strain. Another O157:H7 strain that had been cured of the large plasmid yielded only a band of 224/227 bp (A59). None of the non-O157:H7 *E. coli* strains nor any of the *Shigella* isolates yielded amplicons in the 166 or 224/227 bp range. But some of these non-EHEC *E. coli* strains did yield amplicons in the 300–400 bp range (Figure 1).

## Universal GeneComb Kit Results

The GeneComb kit successfully detected all of the amplicons that were observed via agarose gel electrophoresis. The simplest design employed two assay spots per comb tooth (Figure 1A). The upper spot utilized two probes (MKP1 and MKP2) and successfully detected amplicons of either SLT I or SLT II, or both. The lower assay spot detected the 166 bp plasmid amplicon (MSFPP). A positive response in either or both positions was diagnostic of *E. coli* O157:H7. None of the other *E. coli* or *Shigella* strains tested positive. Although some nonpathogenic stains of *E. coli* (*e.g.* DH5) consistently yielded spurious bands in the PCR reaction, these were not observed when the PCR product was assayed by the GeneComb.

The SLT I and SLT II amplicons are similar in size and indistinguishable via agarose gel electrophoresis. Therefore, it was of interest to know if the GeneComb kit could differentiate between these two amplicons. In this test the upper assay spot contained only the probe for SLT I (MKPI) whereas the lower assay spot contained only probe for SLT II (MKP2). Table 1 shows that the GeneComb kit efficiently differentiated between the two amplicons, successfully revealing those strains that produce one, both or neither of the toxins, including two *E. coli* C600 strains transduced with one or the other of the toxigenic phages.[1,7]

The Universal GeneComb kit proved easy to use and the only special equipment required was a 37 °C incubator. But we did observe that the assays described here could also be performed at room temperature and still provide adequate results.

Table 1. Electrophoretic and GeneComb Results

Results from the 40 *E. coli* and *Shigella* isolates tested.

| Strain | Identifying Information | Electrophoresis Result: Plasmid DNA, (a 166 bp amplicon) | Universal Gene Comb Result: SLT I or SLT II, (a 224/227 bp amplicon) | Plasmid specific amplicon | SLT I or SLT II[a] specific amplicon |
|---|---|---|---|---|---|
| A11 | *E. coli* O157:H7 | + | + | + | + |
| A13 | *E. coli* C600 expressing SLT I (phage 933J) | - | + | - | SLT I only |
| A14 | *E. coli* C600 expressing SLT II (phage933W) | - | + | - | SLT II only |
| A21 | *E. coli* O157:H7 | + | + | + | SLT I and SLT II |
| A23 | *E. coli*, non O157:H7 | - | - | - | - |
| A24 | *E. coli*, non O157:H7 | - | - | - | - |
| A44 | *E. coli*, non O157:H7, | - | - | - | - |
| A45 | *E. coli* O157:H7 | + | + | + | SLT I and SLT II |
| A46 | *E. coli*, non O157:H7, | - | - | - | - |
| A47 | *E. coli* O157:H7 | + | + | + | SLT I and SLT II |
| A48 | *E. coli* DH1 | - | - | - | - |
| A49 | *E. coli* O157:H7 | + | + | + | + |
| A50 | *E. coli* O157:H7 | + | + | + | + |
| A51 | *E. coli* HB101 | - | - | - | - |
| A57 | *E. coli* O157:H7 ATCC #43888, with neither toxin gene | + | - | + | - |
| A58 | *E. coli* O157:H7 | + | + | + | SLT I only |
| A59 | *E. coli* O157:H7 cured of 60 Mda plasmid | - | + | - | SLT I and SLT II |
| A60 | *E. coli* O157:H7 | + | + | + | SLT I and SLT II |
| A61 | *E. coli* O157:H7 | + | + | + | + |
| A62 | *E. coli* O157:H7 | + | + | + | + |
| A64 | *E. coli* O157:H7 | + | + | + | + |
| A65 | *E. coli* O157:H7 | + | + | + | + |
| A71 | *E. coli* O157:H7 | + | + | + | + |
| A78 | *E. coli* O157:H7 | + | + | + | + |
| A79 | *E. coli* O157:H7 | + | + | + | + |
| A86 | *E. coli* O157:H7 | + | + | + | + |
| A98 | *E. coli* O157:H7 | + | + | + | + |
| A112 | *E. coli* O157:H7 | + | + | + | SLT I and SLT II |
| A123 | *E. coli* DH5 | - | - | - | - |
| A124 | *E. coli* O157:H7 | + | + | + | SLT I and SLT II |
| A125 | *E. coli* O126 | - | - | - | - |
| A126 | *E. coli* O111 | - | - | - | - |
| A127 | *E. coli* O55 | - | - | - | - |
| A128 | Shigella boydii | - | - | - | - |
| A129 | S. flexneri | - | - | - | - |
| A130 | *E. coli* O126 | - | - | - | - |
| A131 | *E. coli* O28 | - | - | - | - |
| A132 | *E. coli* O112 | - | - | - | - |
| A133 | *E. coli* O128 | - | - | - | - |
| A134 | *E. coli* O127 | - | - | - | - |

a: A plus sign indicates either SLT I or SLT II, or both, was detected, if a distinction between the two was made the results are clearly indicated. A minus sign indicates that neither SLT I nor SLT II was detected.

## Discussion

Three to four hours was required in order to detect PCR amplicons by agarose electrophoresis, whereas less than an hour was required to detect amplicons by the GeneComb method. Furthermore, if combs were preloaded with the desired probes, assay time could be reduced to 40 minutes.

There were various advantages in the use of the GeneComb kit as compared to electrophoretic procedures as far as detection of amplicons was concerned. First, the GeneComb easily differentiated between the two different SLT amplicons. At 227 and 224 bp in size, these amplicons for SLT I and SLT II were indistinguishable via electrophoresis, but were easily and successfully differentiated by the GeneComb test depending upon the layout of the probes on the comb teeth. To differentiate between the two SLT amplicons, probes specific to each were deposited on the comb in different assay spots. When SLT I and SLT II probes were placed in the same assay spot, amplification of either one or both toxin genes was recorded as a positive result demonstrating that multiple probes can be incorporated into each spot. Such an approach depends on an experimental design wherein detection of one or both DNA sequences (such as the SLTI and SLTII sequences) provides an

acceptable clinical answer. Another advantage of the GeneComb kit was that spurious amplicons were not visualized. These spurious amplicons were generated by the multiplex PCR procedures utilized here, and these extra bands were easily detected by agarose electrophoresis, but not by the GeneComb kit procedure (Figure 1). These extra bands could cause confusion during result interpretation if they were interpreted as positive results, as might be the case by newly trained personnel. Additionally, the GeneComb kit was able to detect very small amounts of amplicon, less than could be detected visually. Generally, one tenth (10 µl) of the PCR reaction was used in either electrophoretic or GeneComb kit tests, and this amount proved more than sufficient for detection purposes. However, when the PCR products were diluted 100-fold, only the GeneComb kit consistently detected the amplicons. Biotinylation of the primers had no effect on the PCR results, and amplicons were generated successfully through the use of nonbiotinylated or biotinylated primers. In summary, these results taken togethe r support several conclusions. The GeneComb kit is simpler and more rapidly used (< 1 hr vs. 3–4 hr) than electrophoretic methods and it is as reliable as electrophoretic methods and, additionally, it eliminates noise due to generation of unwanted or spurious bands. The GeneComb kit can also easily distinguish between amplicons of similar size but different sequence, and it is more sensitive than agarose electrophoresis, consistently being able to detect 100-fold less than the standard assay amount. These observations suggest that the GeneComb kit may be useful in situations where electrophoresis may prove difficult to perform, where amplicons may be of similar sizes, where little amplicon may be generated, or where personnel untrained in molecular methods may be required to interpret the results of PCR tests.

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